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VOL. 15, SEC. A. NOVEMBER, 1937 NUMBER 11

FORCE MEASUREMENTS ON STOP-LOG MODELS[1]

By K. F. Tupper[2]

Abstract

A model of a stop-log emergency dam was built for the purpose of obtaining measurements of the hydrodynamic forces acting on the logs during placement. It is considered that the results are of interest to engineers faced with the design of similar structures. They are accordingly presented in non-dimensional form. The single restriction on the applicability of the results lies in the geometry of the structure where the vertical height of the log is exactly one-sixth the depth of the canal.

Logs of three cross sectional shapes were tested, namely, circular, square, and 2 : 1 rectangular (2 horizontal, 1 vertical). The forces were measured on each of the first four logs to be placed in the stream.

Introduction

Emergency dams are provided at the upper entrance to canal locks leading out of large bodies of water such as lakes. Their function is to shut off the flow of water, which would occur should the lock gates be destroyed by accident. The potential damage which would occur by flooding in the event of such an accident is often enormous, and the cost of an emergency provision for obstructing the flow is justified.

The stop-log type of dam consists of a set of logs of length slightly greater than the width of the canal whose ends fit in a groove or recess in the canal wall, and a special crane which can be swung across the canal and which picks up the logs by an attachment to each end. Heavy weights are carried by the crane immediately above the log and serve to force the log downwards through the water. The logs are constructed of structural steel and are made permeable so that they fill with water.

The design of the hoisting gear, and to a lesser extent that of the logs themselves, is contingent upon a knowledge of, or an assumption of, the forces both vertical and horizontal acting on the log during placement. The horizontal force will determine the friction force (vertical) of the log ends in the grooves as they are moved, and this, added to the vertical component of the hydrodynamic force, constitutes the force which the weights and the crane must be able to overcome in lowering and raising a log respectively.

An utter minimum of design data has been secured from the operation of full-scale structures, because to date no large-sized emergency dam has been

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[1] *Manuscript received September 7, 1937.*
Contribution from the Division of Mechanical Engineering, National Research Laboratories, Ottawa, Canada.

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required to operate under emergency conditions. Obviously a suitable test condition, which means a free discharge through a short length of canal, cannot be obtained.

It might be mentioned in passing that to *guess* the magnitude of the hydrodynamic forces is a matter of virtual impossibility. Indeed it is likely that even the sign of the vertical force will be wrong in a guess, and the magnitude may be seriously in error.

The present model tests, although the results may be affected by scale, and to a slight extent by inaccuracies of representation in the model, are believed to provide a useful guide to designers of stop-log emergency dams.

Description of the Apparatus

The apparatus was built for the investigation of a specific problem, and represents a 1/100 scale model of a portion of the Welland canal. Fig. 1 presents a schematic drawing of the equipment as a whole, with the principal dimensions. Fig. 2 is a photograph of the canal model proper, with stop log and balance in place. Fig. 3 is a photograph of the balance assembly and model.

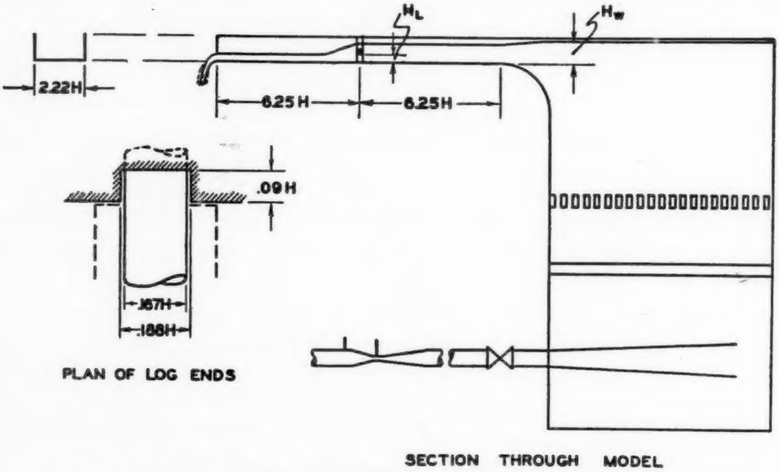


FIG. 1. *Sketch showing general layout of stilling basin and canal model.*

The water supply was obtained from the city mains through a 3 in. pipe line containing a Venturi meter and two gate valves. A stilling basin 6 ft. deep and 2.5 by 3.5 ft. in planform connected the pipe line to the model with a bell mouthed transition section. The stilling basin was fitted with a pair of slat racks and with an expansion cone on the inlet pipe.

The canal model was made of sheet brass and was 0.80 ft. wide and 0.36 ft. deep. The outlet end was fitted with a hinged plate which could be raised; this formed an adjustable weir crest. This was provided as a method of controlling the downstream water level, but was not used.

PLATE I

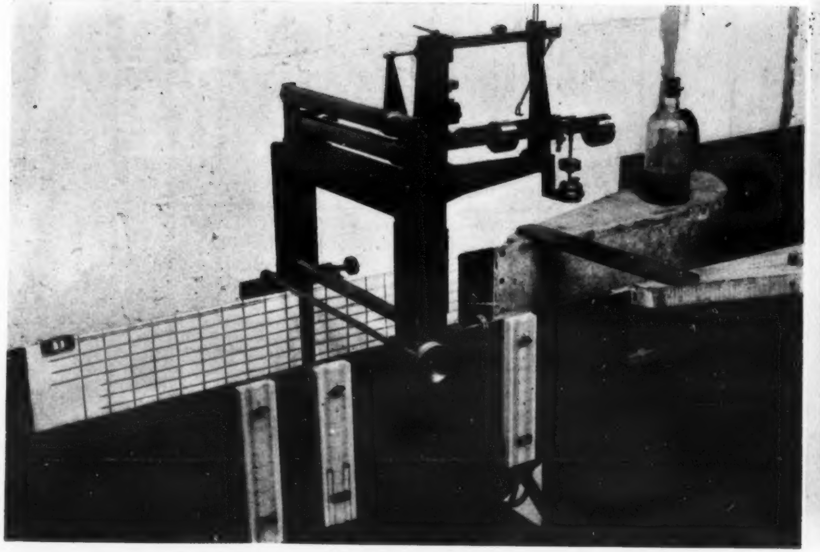


FIG. 2. *View of canal model with balance and circular log in place (elevated).*

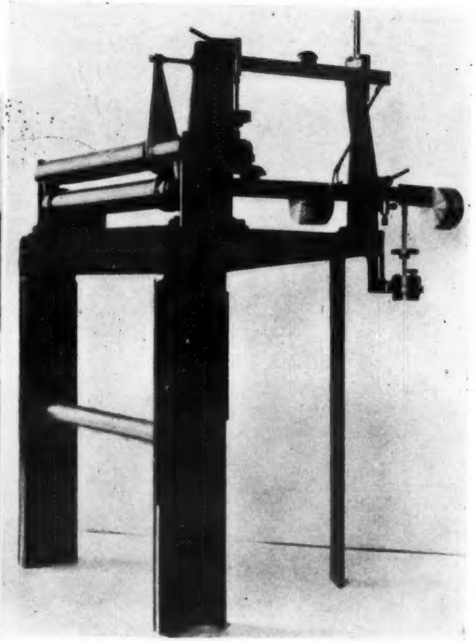
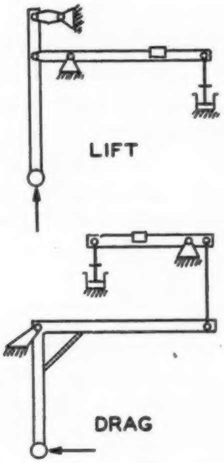


FIG. 3. *View of balance, detached.*

The stop-log models were supported by a balance which was mounted on an inverted U-frame straddling the canal model. The balance was arranged to measure separately the horizontal and vertical components of the hydrodynamic force. The balance could be raised and lowered by a rack and pinion or clamped at any desired height. Fig. 4 is a schematic diagram of the balance mechanism.

A hook gauge was installed in an external chamber to measure the water level in the stilling basin, which constitutes the head of water acting on the model. A number of glass tube manometers were attached to the bottom of the canal model to provide a measurement of the depth of water at various points. The manometers are shown in Fig. 2.



DIAGRAMMATIC ARRANGEMENT OF BALANCE

FIG. 4

Operational Technique

Each series of tests was made with a constant discharge through the model, regulated by means of the valves in the supply pipe line and measured by means of the Venturi meter. This meant that the upstream (stilling basin) water level changed from its nominal value during each test according to the vertical

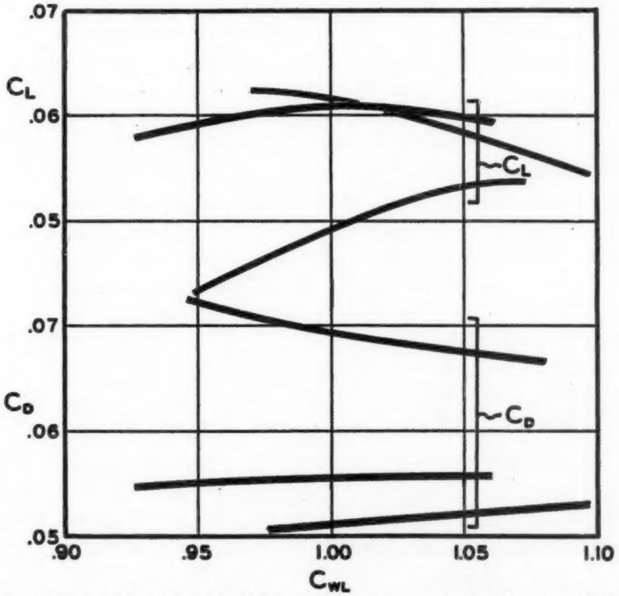


FIG. 5. *Results of tests showing influence of upstream water level upon model forces.*

position of the log. The discharge was so chosen as to give an average value of upstream water level that was approximately correct. This technique was chosen because of its simplicity.

To get an idea of the errors involved because of failure to maintain correct head and to have the correct discharge, a few tests were made with circular logs in the position giving the maximum force, a range of discharges being used. The results are presented in Fig. 5 and show that small variations from the correct discharge cause only negligible variations in the maximum force.

Non-dimensional Coefficients

The results of the force tests are presented in non-dimensional form. Strictly the results are applicable only to exactly similar cases, but because the flow is substantially two dimensional the error will be small for applications to the case in which the dam cross section is exactly similar and the canal width is equal to, or greater than, the depth.

Symbols Used

Dimensional Coefficients

Symbol	*Definition*	*Dimensions*
H	Depth of canal	Length
H_L	Height of log above bottom of canal	Length
H_W	Height of upstream water level above bottom of canal	Length
B	Breadth of canal	Length
Q	Discharge	Length^3 Time^{-1}
L	Lift force (vertical)	Length Mass Time^{-2}
D	Drag force (horizontal)	Length Mass Time^{-2}
g	Acceleration of gravity	Length Time^{-2}
ρ	Fluid density	Mass Length^{-3}

Non-dimensional Coefficients

Symbol	*Definition*	*Derivation*
C_{LH}	Log height coefficient	$\frac{H_L}{H}$
C_{WL}	Water level coefficient	$\frac{H_W}{H}$
C_D	Drag coefficient	$\frac{D}{BH^2\rho g}$
C_L	Lift coefficient	$\frac{L}{BH^2\rho g}$
C_Q	Discharge coefficient	$\frac{Q}{BH^{1.5}g^{0.5}}$

Calculation of Full-scale Forces

Full-scale forces are calculated from the lift and drag coefficients in the following manner.

$$\text{Lift force, } L = C_L B H^2 \rho g$$
$$\text{Drag force, } D = C_D B H^2 \rho g.$$

It may be noted that the factor BH^2 has the dimensions of a volume, and is the scale multiplier; the factor ρg is the weight density of the water (or other fluid to which the experimental results could as well be applied).

Range of Variables Explored

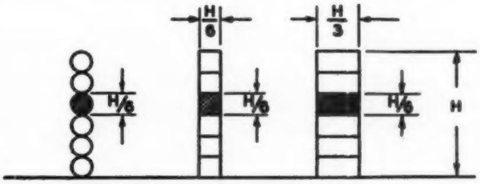
1. Log Height Coefficient

The log height is defined as the vertical distance from the floor of the canal to the bottom of the log under consideration. The lower limit of C_{LH} for each of the first four logs is therefore 0, 0.167, 0.333, and 0.5 respectively. The upper limit of C_{LH} was determined by the flow condition prevailing, since ultimately on raising the log it came quite clear of the stream and experienced no hydrodynamic force. The first log usually came out of the water at C_{LH} about 0.67, and the next three logs each came out at C_{LH} about 0.75.

It should be noted that in gradually *raising* a log the highest value of C_{LH} for which the log is still partly immersed is greater than the value of C_{LH} at which the log first touches the stream when being *lowered*. In the latter case as soon as the log touches the stream a wave is created on the upstream side of the log, and it traverses slowly upstream, ultimately disappearing into the stilling basin. On changing the height of the log in either direction, the hydraulic conditions are transient for a considerable period of time, and it is impossible to make reproducible force measurements. As a consequence all measurements were made under steady conditions which might not be encountered in practice if a log is being moved at all quickly. It is considered that the general magnitude of the forces prevailing under transient conditions is the same as that of the steady forces which were measured; the behavior of the balances did not give any indication to the contrary.

2. Shapes of Log

Logs of three cross sectional shapes were tested, namely;—circular, square, and 2:1 rectangular, the last with its larger dimension horizontal. The shapes are shown in Fig. 6, assembled to form a complete section of a dam.



SHAPES OF LOGS AND DAMS
FIG. 6.

3. *Discharge Coefficient*

As explained above, the discharge, and hence the discharge coefficient, was maintained constant during the tests on each individual log. The discharge coefficients prevailing during the tests are given in Table I.

TABLE I

DISCHARGE COEFFICIENTS

Log	Circular	Square	Rectangular 2 : 1
1st	0.459	0.428	0.418
2nd	0.388	0.357	0.347
3rd	0.286	0.245	0.246
4th	0.173	0.135	0.136

Accuracy of Measurement

Drag

Repeated readings of drag usually agreed to about 1%, but in some cases they differed by as much as 2½%. The drag force appeared to be fluctuating continuously. The measuring technique therefore consisted of an attempt to measure upper and lower limits, and then setting the balance at a reading midway between these and watching to ascertain whether the departures on each side were equally numerous. As a rule the measurement of drag presented no difficulty, the only exception being when a log was just emerging from the stream, and then conditions were quite unsteady.

The drag measurement is not exact owing to a deficiency of the balance used, wherein the pitching moment acts on the drag beam and influences the results. From previous unpublished tests of forces on cylinders of circular and square cross section under similar conditions, it was found that the resultant force never had an eccentricity greater than one-sixth the diameter of the cylinder. On the assumption that this is true also for the rectangular shape, the maximum error introduced on this account in the present tests would be 1½%. The lift measurement is free from this complication.

Lift

The lift balance exhibited wide variations in stability owing directly to the variation in lift with variation in log height. In order to make lift measurements, the log must be free to move slightly in a vertical direction. This amounts to a minute change in log height, and, hence, depending on $\frac{dL}{dH_L}$, to a change in the vertical force. If the slope of the lift curve against log height, $\frac{dL}{dH_L}$, is negative, the balance is stabilized and hence insensitive, but if the slope is positive, the balance is unstable.

As a consequence of this phenomenon the measured lifts, when $\frac{dL}{dH_L}$ had large negative values, are subject to considerable error. This is the case when a log is in its lowest position, and all the lift measurements in this position are uncertain. When $\frac{dL}{dH_L}$ had a positive value the lift balance was unstable, and required to be operated in a special manner allowing only very small motions of the log. When this was done the readings were satisfactory.

Scale Effect

The temperature of the water during the experiments varied from 10° to 20° C. The corresponding kinematic viscosities are 1.4 and 1.1×10^{-5} ft.^{2} sec.$^{-1}$. The Reynolds number may be computed using as a length dimension the depth of the water. Let v be the mean velocity at a section, and d the depth. Then $vd = \frac{Q}{B}$ and the Reynolds number, $\mathrm{Re} = \frac{vd}{\nu} = \frac{Q}{B\nu}$. In the present tests $\frac{Q}{B}$ ranged from 0.165 to 0.562 ft.^{2} sec.$^{-1}$, so that the corresponding Reynolds numbers fall in the region between 12,000 and 51,000.

It is believed that the general flow in the model was of a turbulent nature, and that scale effects would not be severe. There is a possibility that the flow past the circular logs might be in the "mixed flow" regime, and that the drag force measurements in particular might be low on that account.

Results

A. Force Measurements

The results of the force measurements are presented in graphical form in Figs. 7 to 12 inclusive as a plot of C_L or C_D against C_{LH}, and in Fig. 13 as a plot of C_L against C_D. The latter presentation constitutes a polar diagram, and a line drawn from the origin to any point on a curve will have the same direction as the resultant force corresponding to that point.

It is interesting to note that the forces are smallest for the circular shape and largest for the 2 : 1 rectangular. The vertical forces are particularly large for the 2 : 1 rectangular shape. The inclination of the resultant force varies from about +55° to −50°.

B. Flow over Partial Dam

The shape of the water profile is shown in the photographs assembled in Figs. 14 and 15. The grid of lines on the canal wall was intended for the specific case under investigation and represents 5- and 10-ft. intervals full-scale. In non-dimensional form the spacing of the horizontal and vertical

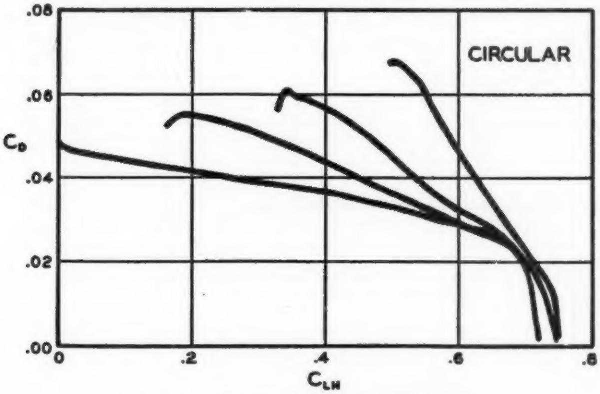


FIG. 7. *Results of force measurements.*

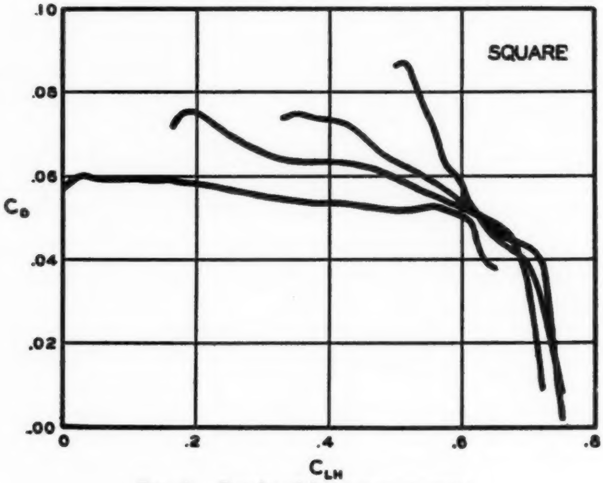


FIG. 8. *Results of force measurements.*

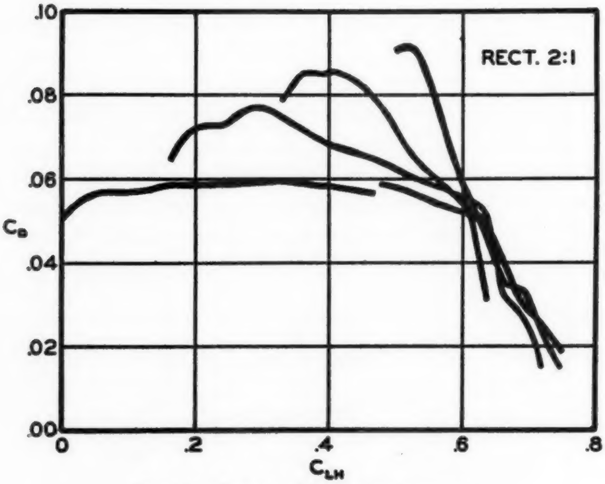


FIG. 9. *Results of force measurements.*

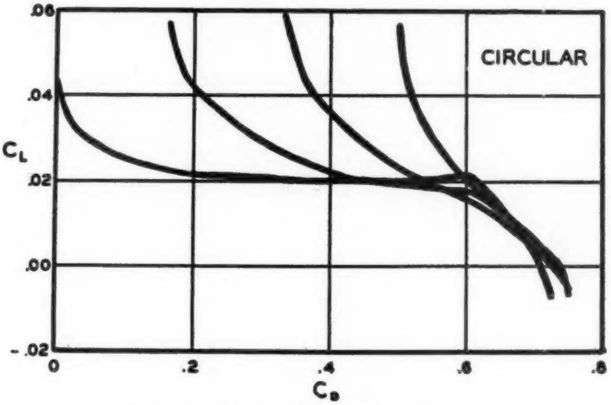


FIG. 10. *Results of force measurements.*

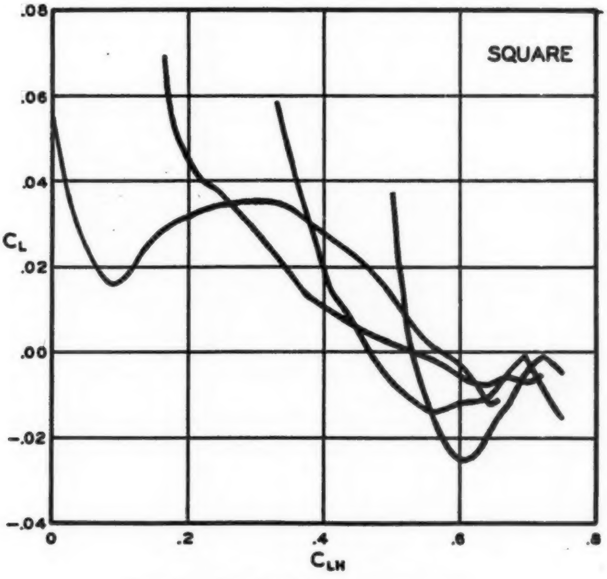


FIG. 11. *Results of force measurements.*

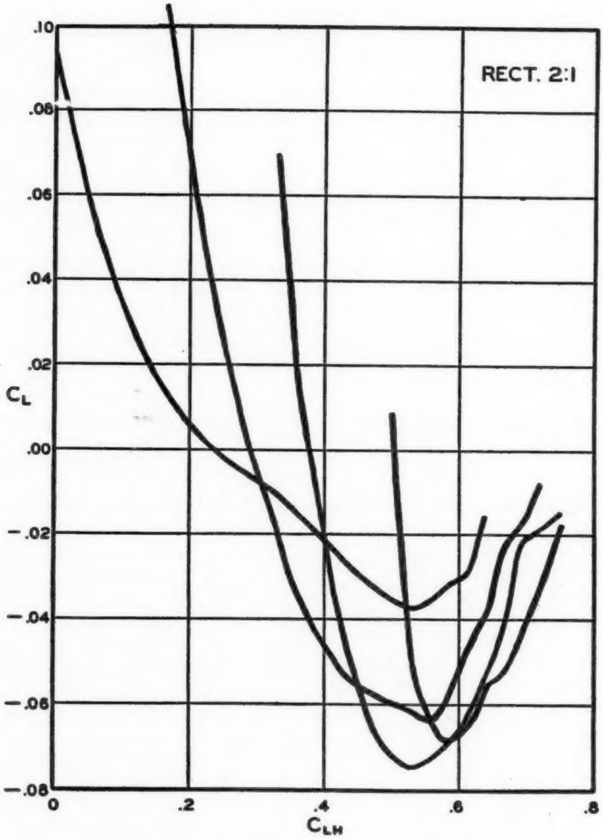


FIG. 12. *Results of force measurements.*

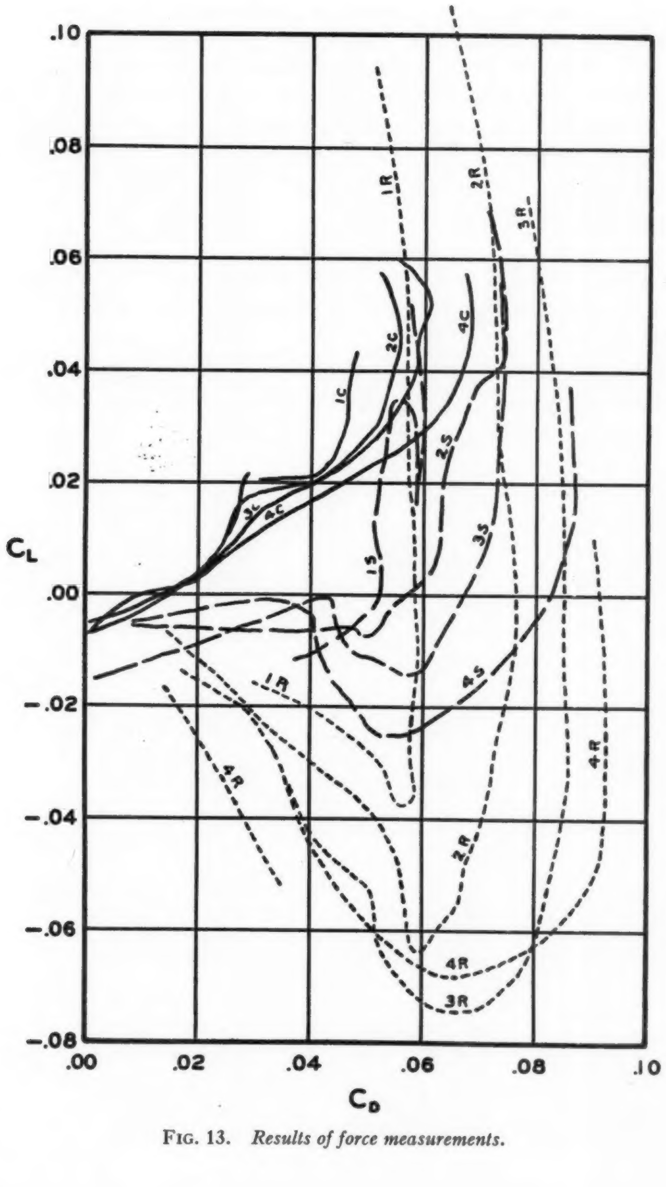


FIG. 13. *Results of force measurements.*

lines of the grid is $\frac{5}{36}H$ and $\frac{10}{36}H$ respectively. In general, the flow past the partial dam is characterized by a change in surface water level, corresponding approximately to the change from maximum potential to maximum kinetic energy. The nature of the flow is undoubtedly influenced greatly by the downstream conditions, and if the distance to the free fall from the dam were very large, or if an obstruction existed between them, an hydraulic jump would be likely to occur. Such a jump was easily produced by raising the hinged bottom of the canal model, or by introducing an obstacle downstream from the dam.

Conclusion

It is considered that the force measurements recorded above are sufficiently reliable to be used for engineering design purposes, in the absence of any other data.

The disadvantage of the 2 : 1 rectangular shape from the point of view of the hydrodynamic force is to be noted, and the superior characteristics of the circular shape deserve special attention.

PLATE II

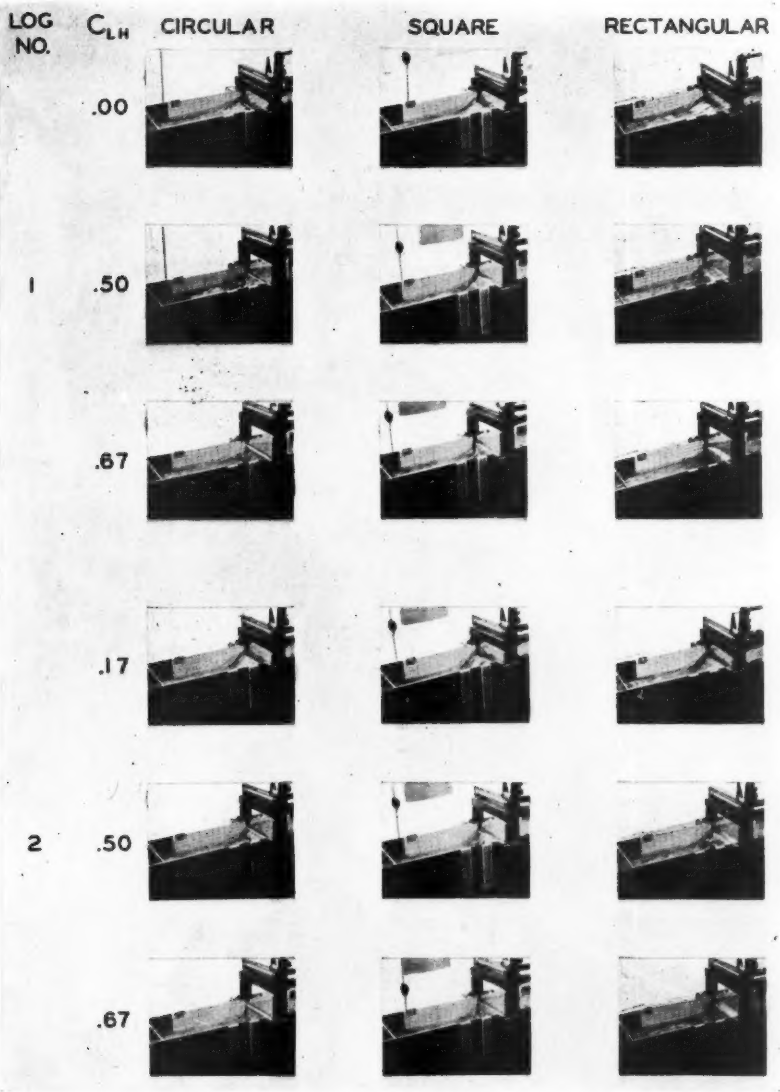
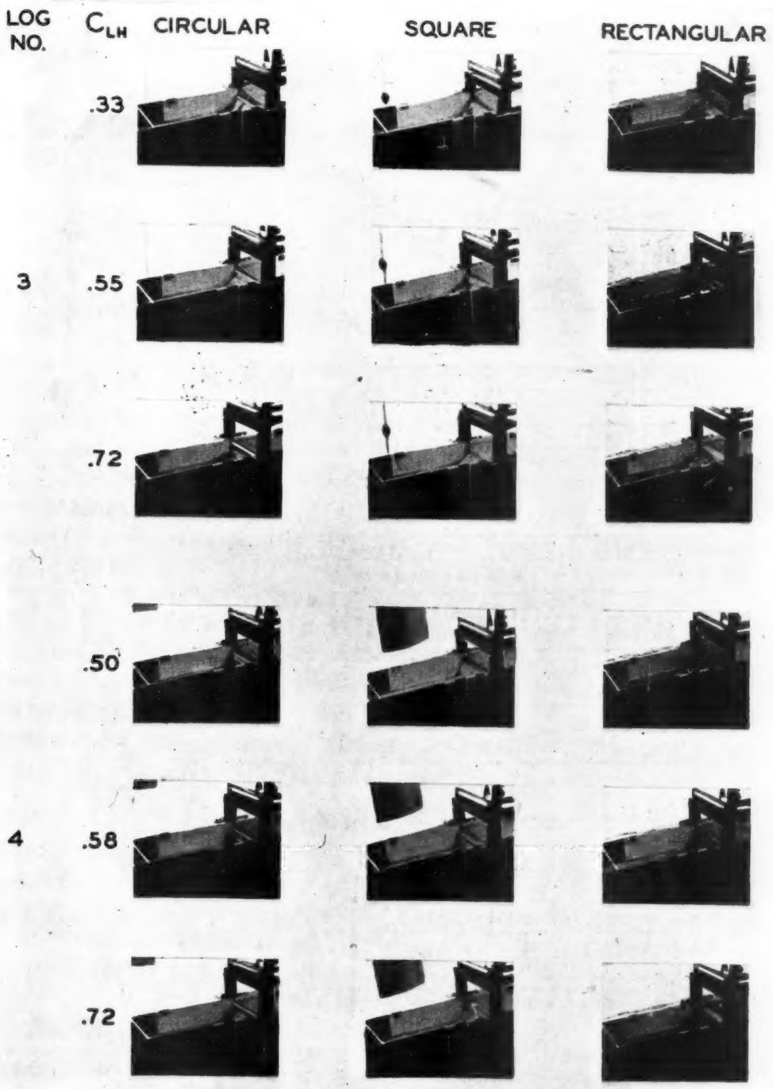

LOG NO.
C_{LH}
CIRCULAR
SQUARE
RECTANGULAR
.00
1
.50
.67
.17
2
.50
.67

FIG. 14. *Views showing flow of water over log and partial dam.*

PLATE III


LOG NO.
C_{LH}
CIRCULAR
SQUARE
RECTANGULAR
.33
3
.55
.72
.50
4
.58
.72

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THE HEATS OF WETTING OF CELLULOSE BY ALCOHOLS AND THEIR AQUEOUS SOLUTIONS[1]

BY J. L. MORRISON[2], W. BOYD CAMPBELL[3], AND O. MAASS[4]

Abstract

The heats of wetting of dry cotton cellulose by several pure and aqueous alcohols have been measured for the first time. The rate at which the wetting occurred was also determined. The results indicate a preferential sorption of water from the aqueous alcoholic solutions. This conclusion is supported by similar measurements in experiments in which absolute alcohols were allowed to wet cellulose containing various amounts of previously adsorbed water.

Water adsorption by standard cellulose has been thoroughly investigated by Argue and Maass (1). They determined the heat of wetting by water of standard cellulose and such cellulose containing varying amounts of previously adsorbed water. The present paper describes an investigation of the adsorption of alcohols and aqueous alcoholic solutions under similar conditions.

So far as is known, the heats of wetting of cotton cellulose by pure alcohols have never been determined. Sheppard and Newsome (6) determined the heats of wetting of cellulose acetate by some alcohols and aromatic hydrocarbons. They found such values as 6.01 cal. per gm. for water, 7.52 for methyl, 5.65 for ethyl, and 4.94 for normal propyl alcohols, and 7.57 for benzene. The specific effect of the acetate groups in such measurements limits speculation as to the possible values for heat of wetting of cellulose itself by the same liquids.

A lone value for the heat of wetting of cotton by a 50% ethyl alcohol solution was reported by Chilikin (2).

Experimental Technique

The apparatus employed consists of a small rotating adiabatic calorimeter, the details of which have been described by Argue and Maass (1). The only change in procedure was the method of filling the inner calorimeter vessel with the dry cellulose. The cellulose after being placed in this vessel was dried in an oven at 100° C. in a stream of dried air. It was then transferred while hot into a metal container (containing phosphorus pentoxide) in the side of which a rubber glove had been cemented. The greased lid could then be adjusted after the cellulose and its silver vessel had cooled down. A ground glass window sealed the top of the metal container.

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[1] *Manuscript received August 27, 1937.*
Contribution from the Department of Physical Chemistry, McGill University, Montreal, Canada. This investigation was carried out in co-operation with the Forest Products Laboratories of Canada, Montreal, and formed part of the research program of that institution.

[2] *Research Fellow, Canadian Pulp and Paper Association, Forest Products Laboratories, of Canada, Montreal.*

[3] *Consulting Physical Chemist, Forest Products Laboratories of Canada.*

[4] *Macdonald Professor of Physical Chemistry, McGill University, Montreal, Canada.*

The cellulose used in the present investigation was prepared by the method employed by Argue and Maass (1). Absolute methyl alcohol (Merck) and absolute ethyl alcohol were used without further drying. Absolute normal propyl and isopropyl alcohols were prepared from samples of 98 to 99% purity. These were refluxed over quicklime for four hours, distilled, and refluxed over magnesium metal for another four hours. Finally, they were distilled from the magnesium powder into dry vessels. Aqueous alcohol solutions were prepared from pure alcohols and distilled water. The percentage compositions were determined by density measurements, made with a hydrometer that measured specific gravity to within 0.002.

Results

The heats of wetting of standard cellulose by absolute methyl, ethyl, normal propyl, and isopropyl alcohols and several water solutions of these alcohols, are given in Table I. In the calculation of the heat evolved, it was necessary to correct for the evaporation of the vapors into the inner calorimeter. The partial vapor pressures and the heats of vaporization of each constituent, at 25° C., were used to make this correction (4, p. 290; 5, p. 138). Further, the best obtainable data on the specific heats of these solutions and pure substances were employed (3, 5, p. 116); in some cases interpolations of the data to 25° C. were necessary.

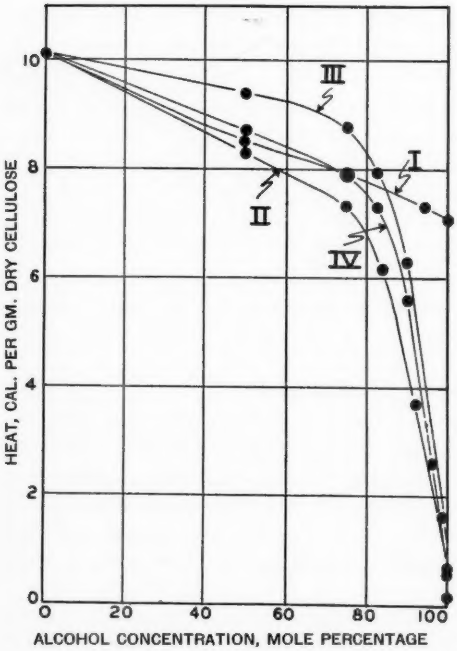

FIG. 1. *Heats of wetting of cotton cellulose by alcohol-water solutions. I, methyl alcohol; II, ethyl alcohol; III, normal propyl alcohol; IV, isopropyl alcohol.*

In Fig. 1 are plotted the data given in Table I. The data are given in mole percentage to facilitate discussion. Absolute methyl alcohol gives a value of 7.07 cal., which is comparable in magnitude to that for water, while the higher alcohols give values less than 1 cal. per gm. of cellulose. The order of the values is the same as the order of molecular weights of the wetting agents.

The quantities of heat evolved in the various methyl alcohol-

TABLE I

HEAT OF WETTING OF STANDARD CELLULOSE BY ALCOHOLS AND ALCOHOL-WATER SOLUTIONS

Alcohol	Concentration, mole % alcohol	Heat of wetting, cal. per gm. dry cellulose			
		Individual determinations			Mean
Methyl	100.0*	7.05	7.12	7.04	7.07
	94.4		7.36	7.26	7.31
	75.0		7.96	7.91	7.94
	50.0		8.49	8.51	8.50
Ethyl	100.0*		0.76	0.65	0.71
	92.6		3.71	3.64	3.68
	84.0		6.13	6.19	6.16
	75.0		7.31	7.42	7.36
	50.0		8.39	8.16	8.27
Isopropyl	100.0*		0.65	0.54	0.59
	96.5		2.57	2.59	2.58
	90.0		5.62	5.54	5.58
	82.5		7.38	7.23	7.30
	75.0		7.93	7.89	7.91
	50.0		8.73	8.65	8.69
Normal propyl	100.0*		0.14	0.18	0.16
	98.75		1.64	1.58	1.61
	90.0		6.22	6.36	6.29
	82.5		7.95	7.92	7.93
	75.0		8.74	8.78	8.76
	50.0		9.43	9.32	9.37

* *Absolute.*

water solutions give almost a straight line from 0 to 100% alcohol. The same is true for all the other alcohol solutions from 0 to 80 mole per cent of alcohol, the slopes over this range being such that normal propyl gives more heat than, isopropyl about the same heat as, and ethyl less heat than, do methyl alcohol solutions. For concentrations of alcohol from 80 to 100%, all but methyl give greatly decreasing heats of wetting, until, at a concentration of 100% alcohol, they give values less than 1 cal. per gm. of dry cellulose.

In conjunction with the above data is given the time to three-quarter value of the heat evolved, for virtually all these solutions, when they wetted the standard cellulose. No values are given for absolute ethyl, normal propyl, and isopropyl alcohols, the heat values themselves being so small that a time curve was difficult to even estimate. The time values were obtained by plotting the temperature difference from the initial temperature (ohms in this case) against the observed time after initiating the wetting process (by starting rotation). The final temperature reading minus the initial temperature reading, allowance being made for heat of rotation, is taken as the total heat of reaction, and the time to three-quarters of this temperature change is taken as the time to three-quarter value of the heat evolution. The equation

$$\text{Heat}_{\text{(final)}} - \text{heat}_{(\text{time } t)} = K \log t,$$

was found to hold fairly well, and the times to three-quarter values may be considered as a measure of the reaction velocity constant.

The results of the time values are given in Table II and Fig. 2. They can be considered as a measure of the time required to totally wet, so far as is possible, the cellulose.

TABLE II

TIME TO THREE-QUARTER VALUE OF THE HEATS OF WETTING OF STANDARD CELLULOSE BY ALCOHOL-WATER-SOLUTIONS

Alcohol	Concentration, mole % alcohol	Time to three-quarter-value, min.	Alcohol	Concentration, mole % alcohol	Time to three-quarter-value, min.
Methyl	100.0*	4.7	Isopropyl	96.5	10.7
	94.4	4.2		90.0	9.0
	75.0	3.4		82.5	5.8
	50.0	3.3		75.0	5.0
				50.0	3.8
Ethyl	92.6	14.0			
	84.0	7.7	Normal propyl	98.75	14.0
	75.0	4.9		90.0	6.8
	50.0	3.5		82.5	5.3
				75.0	4.8
Water		3.1		50.0	4.0

* *Absolute.*

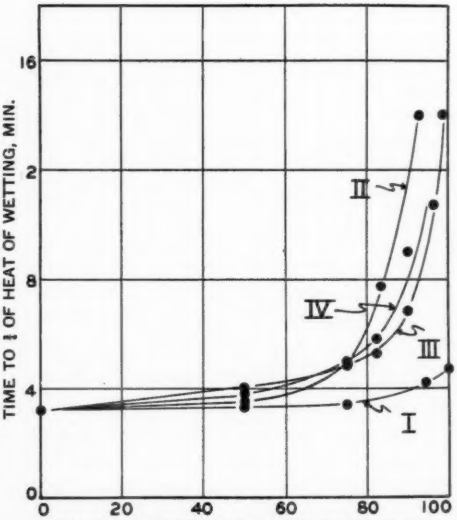

FIG. 2. *Time to three-quarter value for the heats of wetting of cotton cellulose by water-alcohol solutions. I, methyl alcohol; II, ethyl alcohol; III, normal propyl alcohol; IV, isopropyl alcohol.*

Absolute methyl alcohol required one and one-half as much time as water, but the other alcohols require much longer times, to wet the cellulose. Especially is this so with ethyl alcohol. Solutions of the alcohols higher than methyl in the series begin to show rapidly increasing time for wetting at approximately 75 to 80 mole per cent alcohol. This is interesting, as it will also be observed that the values for the heat of wetting for the same solutions begin to decrease rapidly at this concentration.

Several desorption experiments were carried out. Standard cellulose was allowed to absorb definite amounts of water up to 7 or 8% moisture content.

The samples so treated were placed in the inner container of the calorimeter, the lid was adjusted, and a weighed amount of absolute ethyl, normal propyl, or isopropyl, alcohol was placed in the calorimeter proper. Rotation caused contact of the moist cellulose with the absolute alcohol. With virtually all the alcohols a definite cooling took place, the amount depending on the original moisture content of the cellulose. The results of these experiments are given in Table III.

TABLE III

HEAT OF REACTION OF ABSOLUTE ALCOHOLS ON CELLULOSE CONTAINING ADSORBED WATER

Alcohol (absolute)	Moisture content, % dry cellulose	Heat evolved, cal. per gm. cellulose
Ethyl	6.34	−2.48
	6.25	−2.42
	3.83	−2.00
	1.60	−0.49
	0.41	+0.23
	0.00	+0.71
Normal propyl	7.45	−5.06
	2.66	−2.35
	0.00	+0.16
Isopropyl	6.50	−5.56
	2.80	−2.52
	0.00	+0.59

Discussion

The heats of wetting of cellulose by the pure liquids are in the order:—water, then methyl alcohol with a relatively small difference between these two, followed by a big decrease to ethyl, isopropyl, and finally normal propyl alcohol. The values for the last three are of a similar small magnitude. This is the order in which the heats might be expected to vary, but unfortunately data on adsorption of all these compounds by cotton are not available. When these are obtained, it will be of interest to compare molecular amounts adsorbed at the same relative humidity with the heats of adsorption and some of the known thermal constants of these compounds.

Aqueous solutions of methyl alcohol yield virtually a straight line relation for concentrations between 0 and 100% alcohol. The heats of wetting of water and of absolute methyl alcohol are of similar magnitudes. Hence, the affinities of cellulose for these two liquids are similar. Any mixture of the two would be expected to give an average heat of wetting that would depend on the relative amounts of each present; this is found to be true.

Further, the time for the wetting by methyl alcohol, though one and one-half as much as that by water, is quite comparable to the time for water. Solutions of methyl alcohol and water give intermediate values for the time, though there is some tendency for the time value for pure water to be approached quite rapidly.

Aqueous ethyl, normal propyl, and isopropyl alcohol solutions do not give a straight line relation between the quantity of heat evolved by the absolute alcohol and that by water. They behave similarly, however, in that from concentrations of 0 to 80 mole per cent alcohol, their solutions give a straight line relation of heat magnitudes, comparable to that for methyl alcohol solutions. For concentrations greater than 80 mole per cent alcohol they all show low values of heat evolution. These facts require some explanation.

Consider a cellulose fibre wetted with an aqueous alcohol solution. The heat evolved by each constituent ought to depend merely on the volume, or perhaps on the mole per cent of each present. But this certainly is not the case for solutions of alcohols higher than methyl. In fact, a very low water concentration gives a quantity of heat considerably greater than that evolved by absolute alcohol. Certainly there must be a preferential adsorption of the constituent giving high evolution of heat; namely, water.

Probably at all concentrations there is some preferential adsorption of water at equilibrium. If the concentration of water in the alcohol is greater than 20 mole per cent, there is sufficient water immediately available for such equilibrium to be attained quickly. But if the water concentration is less than this, there is a time lag caused by the slowness of diffusion of the water to the cellulose surface. The effect is intensified as the proportion of water is reduced.

The possibility that water is preferentially adsorbed from the alcohol solution, at least for alcohols higher in the series than methyl, invites some further discussion. If water is preferentially adsorbed, then a process the reverse of solution in alcohol is occurring. These concentration changes should involve appreciable heat changes, which would be involved in the measurements of the heat of wetting of cellulose by the alcohol solution.

When ethyl alcohol is mixed with water at 25° C., heat is evolved over the whole range of concentrations (5, pp. 159, 160). Hence, the reverse process, namely, the removal of water from ethyl alcohol, would result in heat being absorbed (cooling). When water and normal propyl alcohol are mixed at 25° C., then over the range of concentration 35 to 100% alcohol, heat is absorbed (5, pp. 159, 160) (with ethyl alcohol the reverse is true). Therefore, on removing water from a normal propyl alcohol solution, heat evolution (warming) would occur.

Now consider the relative magnitudes of the heats of wetting of solutions containing the same mole percentage of these two alcohols. Normal propyl alcohol solutions give the higher values of the two; in fact, ethyl alcohol solutions give the lowest values of all. These facts definitely favor the assumption of a preferential sorption of water. They do not indicate which alcohol loses the most water. The fact that normal propyl alcohol solutions give higher values does not show that more water is sorbed from this alcohol, since the concentration change itself would cause a warming, while the reverse is true for ethyl alcohol. But some other evidence may indicate the relative affinities.

In the series, water, ethyl, and normal propyl alcohols, one would expect a greater affinity of water for ethyl than for normal propyl alcohol. Now if cellulose is added to these systems, it will sorb water from either alcohol—and probably more readily from normal propyl alcohol. The higher heats evolved by the normal propyl alcohol solutions would support this conjecture if it were not for the interfering factor of concentration changes. However, the time curves definitely indicate that normal propyl alcohol solutions reach their final heat values sooner than ethyl alcohol solutions of high alcohol concentration. This would indicate that water is more readily removed from normal propyl alcohol; that is, the relative affinity of water for ethyl alcohol is greater than that for normal propyl alcohol.

For the present, some explanation has been offered for the observed phenomena when alcohols and alcohol-water solutions wet cellulose. Further speculations were made possible by the results of desorption experiments in which absolute alcohols were allowed to react with samples of cellulose already containing adsorbed water.

The data of these desorption experiments were given in Table III, and some very interesting calculations were made from these in combination with other data. The other data are heats of solution of water in alcohols (5, pp. 159, 160), heats of wetting of cellulose by aqueous alcohols (Table I), and Argue and Maass's heat of adsorption values of water by cellulose (1).

With these data it is possible to calculate the result of either of two processes that have the same initial conditions and should have the same final conditions by different sets of intermediate steps.

Consider a hypothetical three-compartment calorimeter. In one compartment is 1 gm. of dry cellulose, in another a small amount of water corresponding to the amount sorbed on cellulose in the desorption series, and in the third a fairly large definite amount of absolute alcohol.

Process 1

The water compartment is first connected with the dry cellulose. This would involve the evolution of heat as given by Argue and Maass's adsorption data (1, p. 568), in amount depending on the percentage of water relative to cellulose. Call this h_1.

Then allow the alcohol from the other compartment to make contact with the moistened cellulose. The heat evolved in this operation has been measured in the desorption experiments whose results are given in Table III. Call this heat h_2.

Now both water and alcohol are in contact with cellulose.

Process 2

The water and alcohol compartments are connected first. This causes the evolution of the heat of solution of the water in the alcohol, a value that may be obtained from the literature (5, pp. 169, 170). This may be called h_a.

Then permit the above mixture to wet the dry cellulose in the other compartment. The heat involved in this operation has been measured in the present work on heat of wetting as given in Table I, and it is called h_b.

Since the same final condition is reached from the same initial condition in the two processes, the net heat evolution should be the same in the two cases, *i.e.*,

$$h_1 + h_2 \text{ should } = h_a + h_b.$$

Table IV shows that such agreement is not obtained.

TABLE IV

CALCULATIONS CONCERNING TWO POSSIBLE PROCESSES

Original moisture content—% cellulose	Heats evolved, cal. per gm. of cellulose					Water, %
	h_1	h_2	h_a	h_b	$(h_1 + h_2) - (h_a + h_b)$	
Ethyl alcohol						
6.34	7.90	−2.48	0.49	1.00	3.93	2.20
6.25	7.87	−2.42	0.50	0.94	4.01	2.26
3.83	6.19	−2.00	0.33	0.86	3.00	1.60
1.60	3.20	−0.49	0.15	0.76	1.80	0.92
0.41	0.95	+0.23	0.03	0.72	0.43	0.18
Normal propyl alcohol						
7.45	8.26	−5.06	−1.02	1.32	2.90	1.40
2.66	4.80	−2.35	−0.43	0.62	2.26	1.16

The heat evolved from Process 1 is always greater than that in Process 2, and some explanation of this is required.

Possible Explanations

(a) The heat of wetting of aqueous alcohol on cellulose, h_b, should be larger. In the case of Process 1, the water may open up a larger surface (making it available for wetting by the alcohol solution) than would obtain when the aqueous alcohol alone is initially wetting the cellulose as in Process 2. If reference is made to Table IV, it is seen that h_b for ethyl alcohol is 1.00 cal. for the sample containing 6.34% moisture, and, to account for the observed difference of 3.93 cal., an additional surface nearly four times as large as the existing one would have to be formed. This seems too large to be reasonable, though some such action may take place and partly account for the results.

(b) The value h_2 of Process 1 may be too small. It is quite evident that the cooling, when absolute alcohol is sorbed on moist cellulose, is due to a removal of at least some of the adsorbed water. It is possible that all the water may not be desorbed, and low values of h_2 may result.

If this explanation is correct, then a first approximation to the amount of water continuously held on the sample may be calculated by comparing the heat differences between the two processes with the heats of sorption found by Argue and Maass (1). A still closer approximation may be made by making due allowance for this in calculating corrected values of h_a and h_b. It is found that this correction is very small. The amount of water so calculated is shown in the final column of Table IV.

(c) It is possible that h_b is too small—but not for the reason put forward in Explanation *(a)*. Evidence has been given to show that water is preferentially sorbed on cellulose from aqueous alcohol solutions. The time curves seem to indicate that this preferential sorption is slow from alcohol solutions of low water concentrations. It is possible that water is not as completely sorbed as may be possible if infinite time were allowed. This would lead to low observed values of h_b.

At present, Explanation *(b)* seems more reasonable, but the question now arises as to the mechanism leading to the observed state of affairs.

The first thing that seems to come to mind is that it is a time effect, and that if infinite time were given, water could be taken away as in Process 1 and added, as in Process 2, until Process 1 = Process 2. If there is such a time effect then a similar consideration may apply to both Explanations *(b)* and *(c)*. In *(c)*, infinite time would allow more water to add on to the cellulose, and in *(b)*, the same would allow more water to be removed from the cellulose. It is possible that both *(b)* and *(c)* operate simultaneously, and that the two processes (1 and 2) would in time become equal. The final amount of water left on the cellulose would be some intermediate percentage, of the same magnitude whether the water was adsorbed or desorbed. The true equilibrium process would then be represented by some line intermediate between those for Processes 1 and 2 as given in Fig. 3.

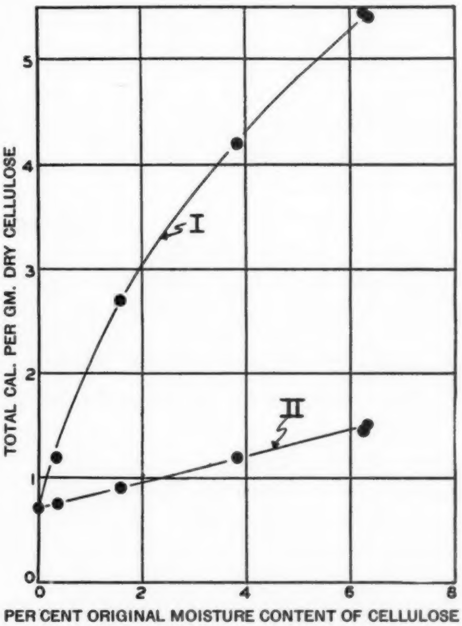

FIG. 3. *Two processes for addition of water and ethyl alcohol to cotton cellulose. I, Process 1, (a) water plus dry cellulose, (b) then plus alcohol; II, Process 2, (a) water plus alcohol, (b) solution added to dry cellulose.*

This speculation has no experimental evidence to support it, because definite end values seemed to have been reached in reasonable times; and until some experiments are carried out in which measurements are made for hours or days, it cannot be proved.

Another mechanism is possible. Water may be able to enter places that alcohol cannot reach; and hence the water might be left there indefinitely. But, evidence from this laboratory seems to indicate that alcohol can replace all the water from cellulose if the alcohol is constantly renewed; it shows that wherever water penetrates, alcohol also can, providing that water was there first. Hence, it may be only a matter of waiting for diffusion to take place—this diffusion into the small interstices likely present in cellulose would require a long time.

Although the amount of water held by cellulose after alcohol contact (according to Table IV) is lower, almost in proportion to the amount originally present, no substantial conclusions may be drawn from this fact. These amounts are what might be expected as ordinary sorption phenomena, *viz.*, that there is an equilibrium between the amount of water sorbed on the solid and that in solution, and that the ratio of the water on the solid to that in solution is greater when the concentration is small.

Several possible explanations and mechanisms have been given for the anomalous results observed when alcohol is sorbed on moist cellulose. The simplest one advanced, namely, that it is only a question of insufficient time being allowed for complete diffusion, may be the best explanation. However, no attempt is being made to come to too definite a conclusion.

The evidence from the heat of wetting of cellulose by aqueous alcohols seems to indicate quite distinctly a preferential sorption of water. The magnitude of the heat values and the time to reach those values both favor this conclusion.

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THE EFFECT OF SULPHITE LIQUOR COMPOSITION ON THE RATE OF DELIGNIFICATION OF SPRUCE WOOD AND YIELD OF PULP[1]

By J. M. Calhoun[2], F. H. Yorston[3], and O. Maass[4]

Abstract

A technique has been devised for studying the delignification of wood in sulphite liquor under accurately controlled conditions. The rate of delignification of resin extracted spruce wood-meal has been determined at 130° C. over the concentration range 0.2 to 1.4% combined, and 2 to 14% total, sulphur dioxide. The reaction shows a systematic deviation from the first order relation that is the same for any composition of liquor. A quantitative relation between the rate of delignification and the concentration of free sulphur dioxide, or better, the partial pressure of sulphur dioxide gas, has been established. The nature and significance of this relation is discussed in connection with the mechanism of the delignification process. It has been shown that the hydrogen ion concentration alone does not determine the rate of cooking, but evidence is presented that indicates that the product of the concentrations of the hydrogen and bisulphite ions is the controlling factor.

The yield of pulp for any given lignin content is independent of the free sulphur dioxide over the whole concentration range, but increases in a marked manner with the concentration of the combined. An explanation is offered, and the practical aspects are pointed out.

Introduction

The question of liquor concentration is one of the most controversial in the sulphite industry (12). This is due to the fact that the effective liquor concentration in commercial cooks depends more on digester operation than on the initial composition of acid. None of the factors, temperature, pressure, or composition can be maintained constant, and it is therefore impossible to determine the actual relation of liquor concentration to the course of the cooking under mill conditions.

It is generally believed that an increase in free sulphur dioxide increases the rate of pulping, but that the combined sulphur dioxide, in excess of a certain minimum, is of little consequence (4, 14). Up to the present time, no quantitative relations have been established. Stangeland (18) made some small-scale laboratory cooks with constant combined, and different total, sulphur dioxide concentrations, but his results are difficult to interpret because he used low liquor ratios and measured the removal of "incrustants" rather than lignin. McGovern (13) made a series of semi-commercial cooks with liquor containing initially 1.2% combined, and 5 to 20% total, sulphur dioxide. He showed that the rate of cooking increased with the strength of the acid, but to a decreasing extent at the higher concentrations.

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Contribution from the Department of Physical Chemistry, McGill University, Montreal, Canada. This investigation was carried out in co-operation with the Forest Products Laboratories of Canada, Montreal, and formed part of the research program of that institution.

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Miller *et al.* (14) maintain that the "excess" sulphur dioxide (total $-2\times$ combined) governs the rate of cooking. Gishler and Maass (5), on the other hand, have shown, from a study of the equilibria existing in sulphite liquor, that a complex ion corresponding to 2CaO to $3SO_2$ might exist, in which case the true free sulphur dioxide would correspond to the total $-1.5\times$ combined. Mitchell and Yorston (15) obtained some evidence in support of this view by cooking resin extracted sawdust in various sulphite liquors. However, insufficient data were obtained to make the results conclusive.

The object of the present investigation was to obtain reliable and comparable data on the effect of various constituents of sulphite liquor on the rate of delignification and yield of pulp, under such exact conditions that undesirable factors could be eliminated, and without the restrictions imposed by commercial practice. Two purposes were in mind, *viz.*: first, to obtain a quantitative relation between one or more components of sulphite liquor and the rate of pulping, in order to help elucidate the mechanism of the delignification reaction; and second, to determine the practical advantages of cooking with different liquors, and to provide data for the more intelligent study of sulphite pulping under commercial conditions.

Experimental

In order that interpretation of the results would be possible, it was necessary to make small-scale cooks under conditions of constant temperature, pressure, and concentration, and to eliminate the difficulties of penetration and diffusion as much as possible. This was accomplished by using a high liquor ratio, and by using wood-meal instead of chips. That these are conditions impossible in commercial practice, in no way invalidates the results.

The wood used for cooking was well seasoned white spruce of density 0.43. It was ground and sieved to 40 to 100 mesh, and then the resin was extracted with alcohol-benzene (1 : 2) for 12 hr. in a Soxhlet apparatus. It was then air-dried and stored for use.* The lignin content of the prepared meal was 27.5%.

Several preliminary series of cooks were made in the bronze bomb digesters (200 cc.) used in previous investigations (3). However, it was found that the rubber gaskets used in sealing the bombs were attacked by the liquor at cooking temperatures; this resulted in appreciable loss of sulphur dioxide. It was, therefore, necessary to devise a new method.

Pyrex bombs of 100 cc. capacity, 1⅜ by 5 in. with a neck ⅜ by 2 in., were constructed. Six bombs were used for each run. Each bomb was charged with 2 gm. of the wood-meal of a known moisture content. Sulphite liquor of the desired concentration was cooled below 0° C. to minimize loss of sulphur dioxide and injected into the bombs. Sufficient space was left to permit expansion of the liquid at 130° C. Before the tips of the bombs were sealed, the air was flushed out by a stream of sulphur dioxide gas. The liquor ratio was about 50 : 1, ten times that used in commercial practice.

Each glass bomb was provided with an outer metal casing, capable of withstanding high pressures. The space between the bomb and the jacket was filled with water to reduce the pressure difference. The loaded bombs were dropped into a well stirred glycoline bath, preheated a little above 130° C., to allow for the cooling effect of the bombs. The temperature of the bath was then held constant at 130° C., which was the cooking temperature employed throughout this investigation. The bombs were placed on their sides in the bath to prevent accumulation of the meal at one end. At given intervals the bombs were removed from the bath and quenched in water.

After the bombs were cooled below 0° C., they were opened, and the liquor was analyzed immediately by the Palmrose method (16). In spite of the high liquor ratio, the concentration of lime and sulphur dioxide decreased slightly during cooking. Therefore, the mean liquor concentration was taken as the average of that for all the bombs in a given run, analysis being made after cooking. The cooked wood-meal was filtered, washed with tap water, oven dried, weighed, and analyzed for lignin. All lignin determinations were made by the Ross-Potter method (17), and all yields calculated on the resin extracted, bone-dry wood basis.

Correction for the Sulphur Dioxide in the Vapor Phase

As the bombs are heated to 130° C., some sulphur dioxide passes into the vapor phase; this reduces the concentration in the liquid. From a calibration of the bombs, the volume of both liquid and vapor phases at 130° C. could be calculated, allowance being made for expansion of the liquid. The partial pressure of sulphur dioxide for the desired concentration and temperature was obtained by extrapolating the data of Gurd, Gishler, and Maass (7). Thus the loss of sulphur dioxide from the liquid could be calculated and the analytical concentration corrected.

Correction for the Rate of Heating of the Bombs

The observed time of cooking was taken from the moment the bombs were dropped into the hot bath to the moment they were quenched in water. It became apparent, however, that for short, fast cooks, the time required for the bombs to heat up to 130° C. would introduce an appreciable error in the results. Previous investigators have made various rough estimates to correct for this error in timing, or have ignored it completely.

An indirect method was used to determine this correction accurately. One bomb and casing were both fitted with stems so that a thermometer could be suspended in the centre of the glass bomb, while the bomb was immersed in the oil bath. Actual heating curves were then obtained for the following:

(a) Bomb and casing filled with water at 20° C.; bath at 100° C.

(b) Bomb and casing filled with oil at 20° C.; bath at 100° C.

(c) Bomb and casing filled with oil at 20° C.; bath at 130° C.

With the bath at 100° C., the ratio of the times to a given temperature for *(a)* and *(b)* was approximately constant. Hence from *(c)* a fourth curve could be calculated:

(d) Bomb and casing filled with water at 20° C.; bath at 130° C.

This was considered sufficiently close to the actual conditions of a cook with sulphite liquor.

The calculated curve for *(d)* was exponential in shape, the temperature in the bomb rising rapidly at first, but more slowly as the temperature of the bath was approached. It was therefore necessary to determine the amount of cooking that took place during the heating period.

On the assumption of a temperature coefficient of the sulphite process of 2.0 per 10° interval, and a linear change in temperature, the following relation was derived:

$$\theta_1 = \int_0^t 2^{tx/10} dt \tag{1}$$

where θ_1 = equivalent time of cooking at the initial temperature, T_1,

x = rate of heating, degrees per minute,

t = time of heating, minutes.

On integration, Equation (1) becomes

$$\theta_1 = \frac{2^{tx/10} - 1}{0.0693x} . \tag{2}$$

It was desired to know the equivalent time of cooking at 130° C. (θ_2) for the heating period. This was obtained from the relation

$$\theta_2 = \frac{\theta_1}{2^{(130-T_1)/10}} = \frac{2^{tx/10} - 1}{0.0693x \, . \, 2^{(130-T_1)/10}} \tag{3}$$

Since the actual heating curve was exponential, it was divided into five approximately linear sections, and Equation (3) applied to each in turn. In this way, the amount of cooking done during the heating period was calculated in terms of the time required to do the same amount of cooking at 130° C. This is independent of the absolute velocity of delignification, and hence the same time correction can be applied to every cook. The time required for the bomb to reach 130° C. was calculated to be 35 min., and the amount of cooking done during this period was calculated from Equation (3) to be equivalent to 22 min. of cooking at 130° C. Therefore, the time correction is 13 min., or 0.22 hr., which is to be subtracted from the observed time the bombs were in the bath.

No correction was necessary for cooking taking place during cooling after removal from the bath, since the temperature of the bombs dropped so rapidly when quenched in water that cooking was almost instantly stopped.

Results

The data for a typical run are given in Table I to illustrate the calculations. The initial liquor concentration was about 0.5% combined, and 4.5% total, sulphur dioxide. Since some sulphur dioxide is lost in filling the bombs, only the final concentrations are averaged. Table I shows the small but

TABLE I

THE RESULTS OF A TYPICAL COOK (RUN No. 59)

Time in bath (uncorrected), hr.	Combined sulphur dioxide, %	Total sulphur dioxide (uncorrected), %	Total sulphur dioxide (corrected), %	Yield of pulp, %	Yield of crude cellulose, %	Lignin, % of pulp	Lignin, % of original wood
0				100	72.5	27.5	27.5
1.5	0.45	4.20	4.17	71.3	56.7	20.5	14.6
3	0.42	4.18	4.17	60.7	53.4	12.0	7.27
4	0.40	4.15	4.12	53.8	50.4	6.34	3.41
5	0.40	4.08	4.07	50.9	48.9	3.96	2.02
6	0.39	4.05	4.04	49.1	48.2	1.92	0.94
7	0.39	4.02	4.00	47.3	46.7	1.17	0.55
Average	0.41	4.12	4.10				

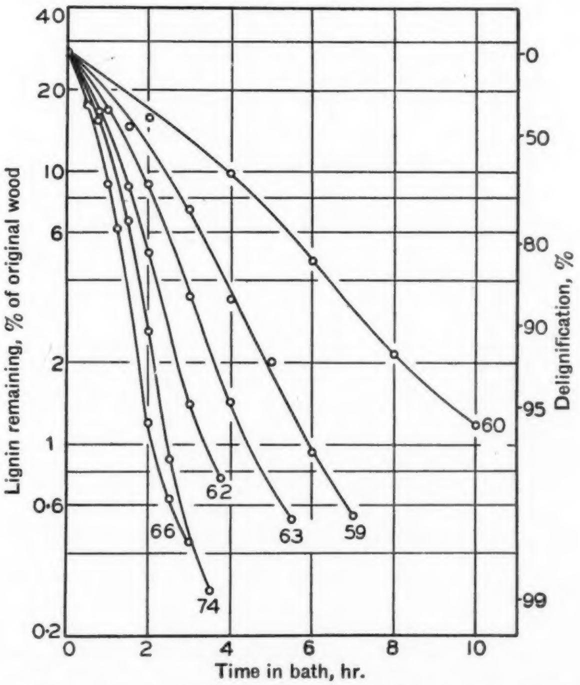

FIG. 1. *Effect of sulphur dioxide concentration on the rate of delignification of spruce wood at 130° C. Series B. Combined sulphur dioxide, 0.4%.*

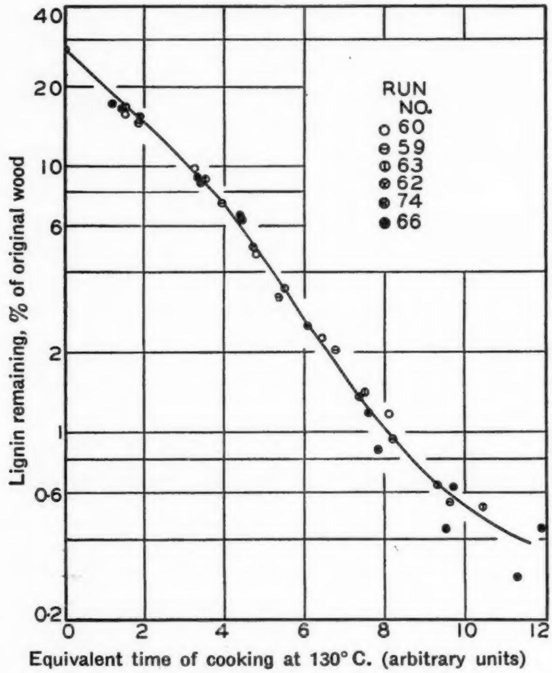

FIG. 2. *Effect of sulphur dioxide concentration on the rate of delignification of spruce wood at 130° C. Series B. Combined sulphur dioxide, 0.4%.*

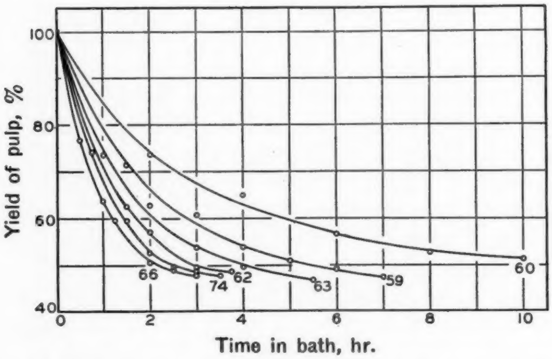

FIG. 3. *Effect of sulphur dioxide concentration on the yield of pulp at 130° C. Series B. Combined sulphur dioxide, 0.4%.*

regular consumption of lime and sulphur dioxide as cooking proceeds. In this particular run, the correction for loss of sulphur dioxide from solution is small, but with cooks at higher concentrations it becomes much more important.

The yield of pulp for Run No. 59 is plotted in Fig. 3, and the lignin as percentage of the original wood is plotted in Fig. 1 on a logarithmic scale. It is seen that the delignification curve deviates somewhat from the first order relation, and hence no attempt was made to estimate velocity constants. The time required to remove 90% of the lignin, as read from the curve, was taken as the best measure of the rate. The yield of pulp at any given lignin content is found by comparing Figs. 1 and 3.

A complete summary of all runs is given in Table II. The range of concentration covered was 0.2 to 1.8% combined, and 2 to 14% total, sulphur dioxide, limited only by solubility restrictions at 130° C. Use was made of

TABLE II

THE EFFECT OF SULPHITE LIQUOR COMPOSITION ON YIELD OF PULP AT 130° C.

Series No.	Run No.	Av. comb. sulphur dioxide, %	Av. total sulphur dioxide (corrected), %	80% delignification		90% delignification		95% delignification	
				Time (uncorr.), hr.	Yield pulp, %	Time (uncorr.), hr.	Yield pulp, %	Time (uncorr.), hr.	Yield pulp, %
A	75	0.16	1.70	10.15	52.5	13.50	49.0	17.5	45.7
	72	0.18	3.68	3.80	54.3	4.96	50.2	6.27	47.6
	71	0.18	5.07	2.90	54.1	3.63	50.2	4.75	47.6
	73	0.17	6.92	2.09	54.0	2.70	49.8	3.44	47.9
	Av.	0.17			53.7		49.8		47.2
B	60	0.39	2.57	5.62	57.8	7.48	53.5	9.45	51.0
	59	0.41	4.10	3.40	57.0	4.45	52.5	5.45	50.0
	63	0.40	5.59	2.55	57.5	3.26	52.3	4.05	49.4
	62	0.40	7.34	1.94	57.6	2.48	52.8	3.01	49.5
	74	0.41	9.67	1.61	57.8	1.97	53.1	2.27	50.7
	66	0.42	12.48	1.30	58.5	1.62	54.2	1.92	51.2
	Av.	0.40			57.7		53.1		50.3
C	56*	0.87	3.27	5.10	61.0	6.90	55.6	8.52	53.0
	51	0.91	4.14	3.90	60.8	5.30	56.0	6.60	53.0
	77	0.92	4.87	3.13	62.0	4.16	56.4	5.20	53.0
	76	0.91	5.88	2.50	61.0	3.26	56.1	3.95	53.4
	52	0.93	5.97	2.52	60.6	3.29	56.4	4.13	53.7
	53	0.91	8.05	2.02	60.2	2.50	55.9	3.10	52.7
	55	0.87	10.65	1.62	60.2	2.00	55.7	2.47	52.3
	61	0.92	13.92	1.22	61.0	1.59	55.9	1.88	53.1
	Av.	0.90			60.8		56.0		53.0
D	67*	1.44	7.68	1.97	63.5	2.53	57.8	3.00	54.8
	68	1.38	10.71	1.57	62.5	1.99	57.2	2.32	54.5
	69	1.39	14.04	1.31	63.0	1.65	57.5	1.92	54.6
	Av.	1.40			63.0		57.5		54.6
E	64*	1.76	11.38	1.45	64.3	1.82	59.0	2.20	55.6
	65*	1.84	13.46	1.27	64.5	1.68	58.5	2.05	55.0
	Av.	1.80			64.4		58.7		55.3

* *Precipitation of calcium sulphite occurred.*

the data of Gishler and Maass (6) on precipitation temperatures, to avoid precipitation of calcium sulphite during cooking. Unfortunately, their temperatures were much too high, a fact that has since been confirmed by Beazley (1). Hence precipitation did occur unexpectedly in several runs (marked with an asterisk, Table II). This made the true concentration at 130° C. uncertain in these cases.

The uncorrected times to 80, 90, and 95% delignification for each run were read from the delignification curves, which for Series *B* are plotted in Fig. 1. The yields of pulp at the corresponding times were read from the yield curves, which for Series *B* are plotted in Fig. 3. The time correction does not affect the yields of pulp taken at a given lignin content.

All the delignification curves showed a similar, definite deviation from the straight line, first order relation. This agrees with the results of Yorston (19) and Corey and Maass (3). It was of interest to know whether the nature of this deviation varied with the liquor composition. To make comparison between the curves in Fig. 1 possible, the time correction was first applied by subtracting 0.22 hr. from the observed times, and then each value was multiplied by the arbitrary factor $6/t_{90\%}$ to make the curves coincident. The result is shown in Fig. 2, and it is seen that all the points fall on the one curve, irrespective of the composition of the liquor, except at low lignin values where analysis is less accurate.

Series *A* and *C* were calculated and plotted in the same way, with the same result. The S-shape of Fig. 2 is quite pronounced, and evidently the mechanism of the reaction, whatever it may be, is the same with all liquors. Increase in sulphur dioxide content does not alter the relative rates of the sulphonation and hydrolysis reactions, which might be expected if Hägglund's theory of sulphite pulping is correct (8, 10).

The Effect of Sulphite Liquor Composition on the Yield of Pulp

An inspection of Table II shows that the yield of pulp at a given lignin content increases rapidly with the combined, but is virtually independent of the total, sulphur dioxide over the whole concentration range. This is brought out in Fig. 4, in which the yield is plotted against the combined sulphur dioxide for different lignin contents.

This effect of the combined sulphur dioxide on the yield is substantiated qualitatively by the results of Hägglund and Nihlen (11) and Mitchell and Yorston (15), although Miller *et al.* (14) state that they could not show any variation in yield attributable to any of the factors in acid composition. It has generally been believed that high sulphur dioxide content resulted in a loss in yield, owing to the greater acidity of the liquor. The contradictions in the literature are no doubt due to lack of uniformity in the methods used and the fact that the yields, in some cases, were not taken at the same lignin content.

The effect of liquor composition on yield may be explained, on the assumption that cellulose degradation is proportional to the hydrogen ion concentration of the liquor at a given temperature. For a constant free sulphur

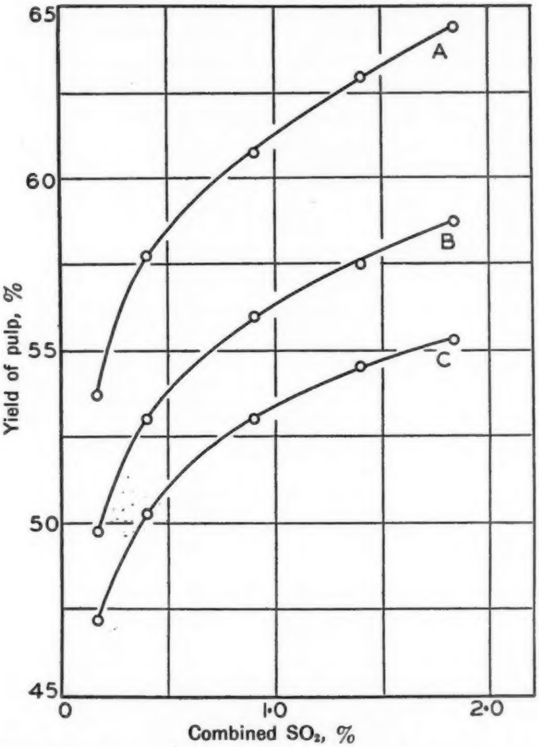

FIG. 4. *Effect of combined sulphur dioxide concentration on the yield of pulp at 130° C. Delignification: A, B, C—80, 90, 95% respectively.*

dioxide content, the pH of the liquor increases with the combined. In fact the pH curves for sulphite liquors of different lime concentrations, obtained by extrapolating the data of Gishler and Maass (5) to 130° C., are almost identical in shape with the yield curves of Fig. 4. That the yield should be independent of the total sulphur dioxide, even though the pH is not, is due to the fact that increased acidity is balanced by the decrease in time required for cooking.

It has thus been shown conclusively that, under the conditions of these experiments, increase in total sulphur dioxide will not affect the yield, whereas an increase in the combined sulphur dioxide greatly improves the yield. The yields obtained with the liquors containing 0.8% or more of combined sulphur dioxide are distinctly better than those realized in mill practice. It may be suggested that the lower yields under mill conditions are due to the low concentration of combined sulphur dioxide prevailing towards the end of the cook. However, opinion is divided as to the effect of changes in the initial concentration of combined sulphur dioxide on the yield from a commercial digester.

The Effect of the Free and Combined Sulphur Dioxide on the Rate of Delignification

The free sulphur dioxide (total − combined) and the "excess" sulphur dioxide (total − 2 × combined) for each run were calculated and are shown in Table III. In Column 8 is given the corrected time to 90% delignification. The rate of delignification, then, is proportional to the reciprocal of this time and is given in the last column of Table III.

TABLE III

THE EFFECT OF SULPHITE LIQUOR COMPOSITION ON THE RATE OF DELIGNIFICATION OF SPRUCE WOOD AT 130° C.

Series No.	Run No.	Av. comb. sulphur dioxide, %	Av. free sulphur dioxide (total − comb.), %	Av. excess sulphur dioxide (total − 2 × comb.), %	pH of liquor at 130° C.	Partial press. sulphur dioxide 130° C., cm.	Time to 90% delignification, hr. (corrected) = $t_{90\%}$	Rate of delignification = $1/t_{90\%}$
A	75	0.16	1.54	1.38	2.35	116	13.28	0.075
	72	0.18	3.50	3.32	2.03	586	4.74	0.211
	71	0.18	4 89	4.71	1.94	414	3.41	0.293
	73	0.17	6.75	6.58	1.86	600	2.48	0.403
B	60	0.39	2.18	1.79	2.53	164	7 26	0 138
	59	0.41	3.69	3.28	2.30	318	4.23	0.237
	63	0.40	5.19	4.79	2.21	466	3.04	0.329
	62	0.40	6.94	6.54	2.12	642	2.26	0.443
	74	0.41	9.26	8.85	2.00	878	1.75	0.572
	66	0.42	12.06	11.64	—	1164	1.40	0.715
C	56*	0.87	2.40	1.53	2.80	178	6.68	0.150
	51	0.91	3.23	2.32	2.66	262	5.08	0.197
	77	0.92	3.95	3.03	2.56	330	3.94	0.254
	76	0.91	4.97	4.06	2.46	425	3.04	0.329
	52	0.93	5.04	4.11	2.45	434	3.07	0.326
	53	0.91	7.14	6.23	2.29	628	2.28	0.438
	55	0.87	9.78	8.91	2.16	875	1.78	0.562
	61	0.92	13.00	12.08	—	1185	1.37	0.730
D	67	1.44	6.24	4.80	—	—	2.31	0.433
	68	1.38	9.33	7.95	—	—	1.77	0.565
	69	1.39	12.65	11.26	—	—	1.43	0.700

**Precipitation of calcium sulphite occurred.*

The rate of delignification is plotted against the free sulphur dioxide in Fig. 5, and against the excess sulphur dioxide in Fig. 6 for the different series of combined sulphur dioxide. Runs Nos. 64, 65, and 67 are omitted because of the uncertainty introduced by precipitation of calcium sulphite. From these curves it is quite clear that the free sulphur dioxide gives the best relation, since there is no variation with the combined except at high concentrations. This will be explained later. Furthermore, if the curve is extrapolated to zero concentration, it is seen that the rate becomes zero for zero free sulphur dioxide.

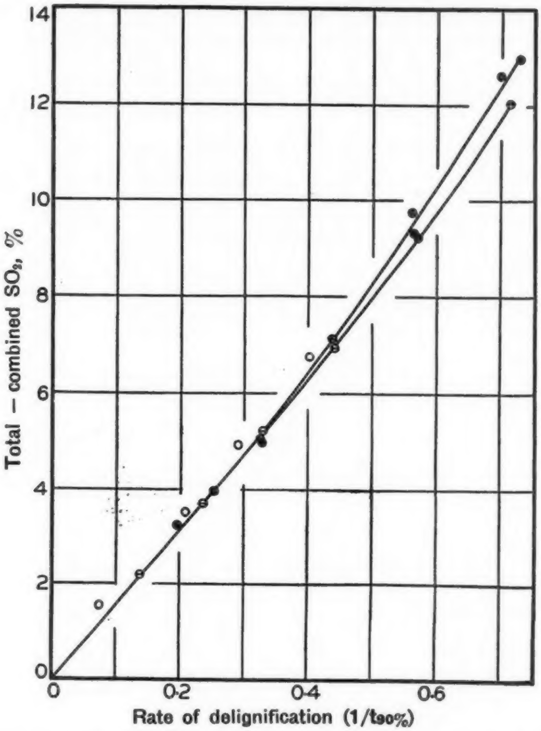

FIG. 5. *The rate of delignification of spruce wood at 130° C. as a function of the free sulphur dioxide.*

Series	○ *A*	⊖ *B*	⊗ *C*	● *D*
Combined SO_2, %	*0.2*	*0.4*	*0.9*	*1.4*

In Fig. 6, on the other hand, the four series of combined sulphur dioxide give distinctly separate curves which cannot be extrapolated to zero rate at zero concentration of excess sulphur dioxide. This proves that the excess sulphur dioxide of Miller *et al.* is not the controlling factor in determining the rate of delignification. If the rate is plotted against the total $-$ $1.5\times$ combined, as suggested by the results of Gishler and Maass (5), a relation is obtained midway between those illustrated in Figs. 5 and 6. No support can be given to such a relation from the evidence of actual cooking obtained here. The ordinary free sulphur dioxide gives the best measure of the rate of cooking, independently of the combined. However, low concentrations of combined sulphur dioxide are possible only at high liquor ratios, since a definite quantity of lime equal to about 5% of the weight of the wood is necessary to neutralize the lignosulphonic acids produced.

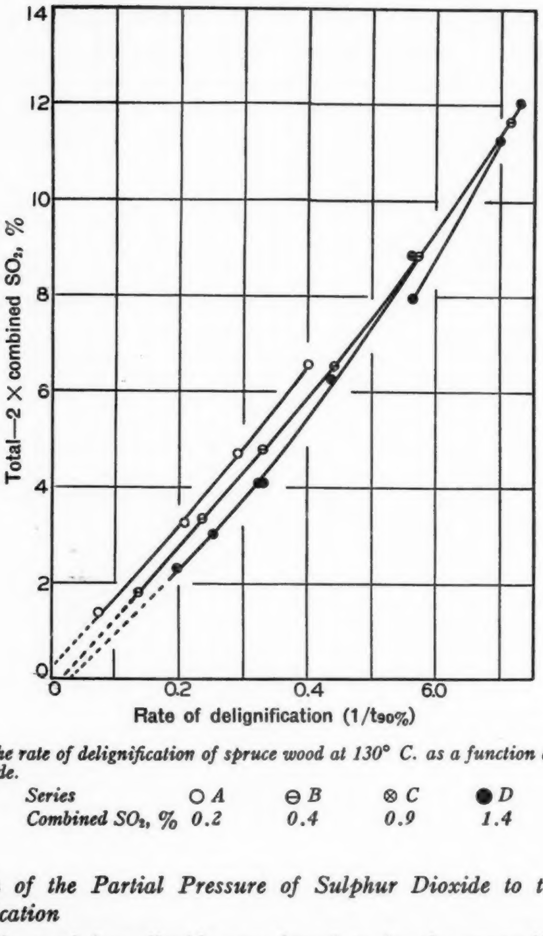

FIG. 6. *The rate of delignification of spruce wood at 130° C. as a function of the "excess" sulphur dioxide.*

Series	○ *A*	⊖ *B*	⊗ *C*	● *D*
Combined SO_2, %	*0.2*	*0.4*	*0.9*	*1.4*

The Relation of the Partial Pressure of Sulphur Dioxide to the Rate of Delignification

Since the free sulphur dioxide was found to be the controlling factor in determining the rate of delignification, further calculations were of interest. The sulphur dioxide in solution that is not in combination with the lime is proportional to the partial pressure of the gas, and since the equilibrium relations between the different ionic and molecular species are so complex, the partial pressure of sulphur dioxide is a better measure of the true free sulphur dioxide than the value calculated from the difference between the total and the combined.

The most accurate measurements of the vapor pressure of sulphite liquor for various concentrations and temperatures have been carried out in this laboratory by Beazley (1). The partial pressure of sulphur dioxide at 130° C.

was obtained for each liquor concentration where data were available. It was necessary to extrapolate to obtain the vapor pressures at concentrations greater than 6% sulphur dioxide, but since the relation is linear except at low concentrations, the extrapolated values are considered accurate.

These partial pressures are given in Table III and are plotted against the rate of cooking in Fig. 7. It is seen that all the points fall on the same curve, irrespective of the combined sulphur dioxide, and that the relation is much more uniform than that given by the calculated free sulphur dioxide in Fig. 5. It is also noticed that Fig. 7 shows a very definite curvature, greater than any experimental error, such that the rate of cooking increases less rapidly at the higher partial pressures.

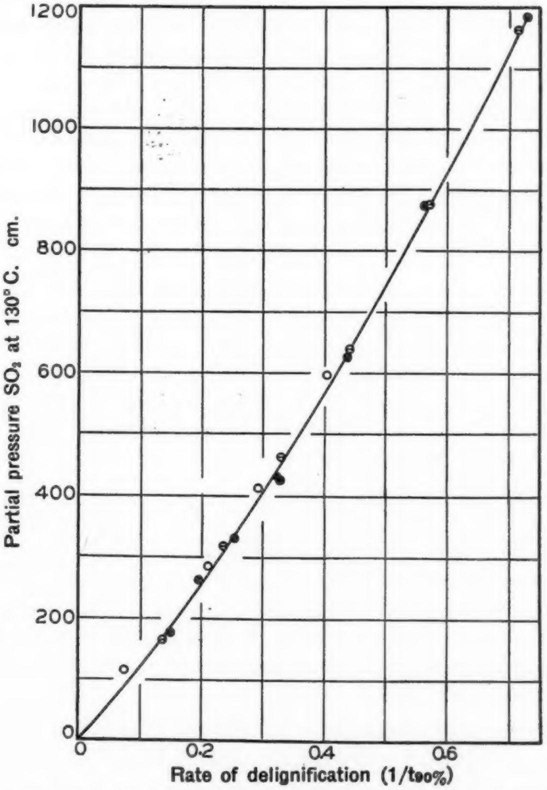

FIG. 7. *The rate of delignification of spruce wood at 130° C. as a function of the partial pressure of sulphur dioxide.*

Series	○ *A*	⊖ *B*	⊗ *C*
Combined SO_2, %	*0.2*	*0.4*	*0.9*

A consideration of the equilibria existing in sulphite liquor is necessary before any further explanation is offered. From a study of the system, calcium-oxide–sulphur-dioxide–water, Gishler and Maass (5) have shown that the following relations hold:

$$P = K_1(SO_2) \tag{4}$$

where P is the partial pressure of sulphur dioxide gas; and

$$(SO_2)(H_2O) = K_2(H_2SO_3) \tag{5}$$

$$(H_2SO_3)K_3 = (H^+)(HSO_3^-) \tag{6}$$

from which it follows that

$$P = \frac{K_1K_2(H_2SO_3)}{(H_2O)} = \frac{K_1K_2(H^+)(HSO_3^-)}{K_3(H_2O)} \,. \tag{7}$$

Equation (7) shows that, provided that the concentration of water is considered constant, the partial pressure of sulphur dioxide is proportional to the concentration of sulphurous acid or the product of the concentration of the hydrogen and bisulphite ions. This relation is quite independent of the presence of lime. The addition of calcium monosulphite to the cooking liquor does not affect the rate of delignification, since the rate is independent of the combined sulphur dioxide, when the free is constant. The decrease in hydrogen ion concentration must be compensated for by the increase in bisulphite ion concentration.

Apart from considerations of the mechanism of the reaction, this suggests that the active cooking agent in sulphite liquor is either sulphurous acid or the hydrogen and bisulphite ions together, and not either ion separately, as has been frequently claimed. This agrees with a statement of Miller *et al.* (14), that the removal of lignin is the result of the specific action of ionized sulphurous acid, but that the effective cooking action could not be attributed to the bisulphite ion alone.

Gishler and Maass (6) have shown that the bisulphite ion in sulphite liquor is strongly buffered and increases only slightly with the concentration of free sulphur dioxide. Increase in the combined, however, causes a large increase in bisulphite. Hence according to Equation (7), an increase in the hydrogen ion concentration by the addition of sulphur dioxide, or an increase in bisulphite ion concentration by the addition of both lime and sulphur dioxide, will increase the partial pressure of the gas as well as the rate of delignification. This explains the relation in Fig. 7.

There are three possible explanations for the curvature observed in Fig. 7:

(a) At the high sulphur dioxide concentrations, cooking is so rapid that diffusion of the lime into the wood-meal may not be fast enough to prevent a local high acidity which might cause slight burning and thus hinder cooking. Some evidence for this was found in a few cases, in which a slight discoloration of the wood-meal was observed at the bottom of the bomb. On the other hand, the relation appears to be much too uniform to be attributed to such

an uncertain cause, and is substantiated qualitatively by the results of McGovern (13), already mentioned.

(b) A second possible explanation may be found by rewriting Equation (7):

$$P(H_2O) = K(H_2SO_3) = K'(H^+)(HSO_3^-) \tag{8}$$

Since the liquors are concentrated, it is not permissible to assume that the concentration of water is constant, as is assumed in the case of dilute solutions. If the cooking action is due to sulphurous acid or the hydrogen and bisulphite ions together, Equation (8) shows that a linear relation with the rate would be obtained only by multiplying the partial pressure of sulphur dioxide by the molar concentration of water. Since this factor is smaller at higher concentrations of liquor, it would tend to straighten the curve in Fig. 7.

An attempt to verify this was made by calculating the concentration of water. No data were available on the density of sulphite liquor in the concentration and temperature range desired. Therefore, the density of sulphurous acid solutions reported by Campbell and Maass (2) was extrapolated to 130° C., and this value used as the closest approximation. It was found that the relation obtained by taking into account the water concentration was slightly closer to the linear than that shown in Fig. 7. However, there was still a curvature greater than the experimental error. It is possible that hydration of the ions present, which would lower the (H_2O) still further, might account for the deviation from the linear relation. This would be impossible to calculate.

(c) A third explanation may be found in Fig. 8, in which the logarithm of the partial pressure of sulphur dioxide is plotted against the logarithm of the rate of delignification. It is observed that an excellent straight line is obtained. This follows the equation of the Freundlich adsorption isotherm and suggests the possibility of an adsorption process. The colloidal nature of lignin has frequently been stressed, and if the concentration of the active cooking agent at the surface of the lignin particles was greater than in the body of the solution, such a relation would hold. That this relation has been found, does not, of course, prove that an adsorption process is involved, but the agreement seems too close to be entirely fortuitous.

The Relation of Hydrogen Ion Concentration to the Rate of Delignification

More information may be obtained from a study of the hydrogen ion concentration of the cooking liquors. It is realized that the presence of the wood may alter the pH slightly, owing to the formation of lignosulphonic acids. However, the very high liquor ratio used will minimize any such change during cooking, so that interpretation of the data is possible.

The actual hydrogen ion concentration of sulphite liquor at cooking temperatures cannot be measured directly. Gishler and Maass (5) have made calculations up to 90° C. from vapor pressure and conductivity measurements. The logarithm of the hydrogen ion concentration plotted against the reciprocal of the absolute temperature gives a straight line. This makes accurate extra-

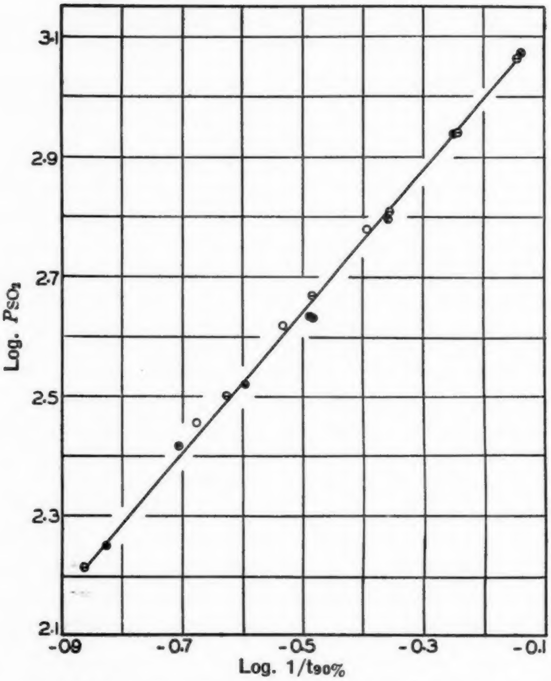

FIG. 8. *The rate of delignification of spruce wood at 130° C. as a function of the partial pressure of sulphur dioxide.*

Series	○ *A*	⊖ *B*	⊗ *C*
Combined SO_2, %	0.2	0.4	0.9

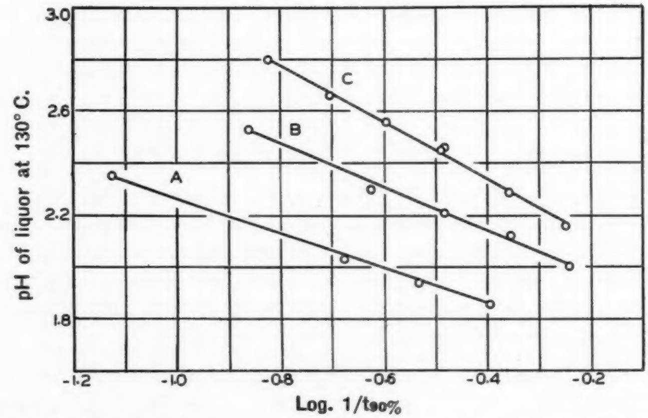

FIG. 9. *The rate of delignification of spruce wood at 130° C. as a function of the hydrogen ion concentration of the liquor.*

Series	*A*	*B*	*C*
Combined SO_2, %	0.2	0.4	0 9

polation to 130° C. possible. A less accurate extrapolation for concentrations above 6% sulphur dioxide was necessary. In this way the actual pH of each liquor was calculated, where possible, and is given in Table III.

In Fig. 9 the logarithm of the rate of delignification is plotted against the pH of the liquor at 130° C. for three series of combined sulphur dioxide. It is observed that a straight line relation is obtained and that the three series give three distinctly separate lines of almost the same slope. The time required for the amount of delignification to increase from 80 to 90% and from 90 to 95% was also calculated. The logarithms of the reciprocals of these times were plotted against the pH of the liquor; this gave relations similar to those of Fig. 9. Here again, three concentrations of combined sulphur dioxide gave three well separated curves. This leads to some rather interesting deductions. Obviously, the rate of cooking cannot be governed by the hydrogen ion concentration alone, or all the points would be on a single line. Evidently the addition of both lime and sulphur dioxide in such proportions that the hydrogen ion concentration is not altered will greatly increase the rate of lignin removal.

This can be explained only on the basis of the theory already outlined, that the cooking action is due to sulphurous acid or the hydrogen and bisulphite ions together, and not to either ion separately. The addition of lime, together with sufficient sulphur dioxide to keep the hydrogen ion concentration constant, increases the bisulphite ion concentration, and hence the rate of cooking. If the combined is held constant, an increase in the free sulphur dioxide increases the hydrogen ion concentration (lowers the pH, Fig. 9), and again increases the rate of cooking.

Discussion

Various theories regarding the mechanism of the delignification of wood in the sulphite process have been proposed. Hägglund (8, 10) considers the reaction as taking part in two stages, *viz.*, first, a sulphonation of the lignin in the solid phase by an addition to some unsaturated linkage in the lignin molecule, and second, an hydrolysis of the sulphonated lignin which separates it from the wood. The rate of the first reaction is supposedly determined by the bisulphite ion concentration and is rapid in comparison with the second. The hydrolysis reaction should then govern the over-all rate of cooking, and would be determined by the hydrogen ion concentration of the liquor.

In support of this theory, Hägglund (9) showed that partially sulphonated wood could be cooked in buffer solutions at a rate that increased with the hydrogen ion concentration of the buffer. Yorston (21) made similar two stage cooks, and found that the rate of removal of lignin in the buffer solution depended on the initial sulphur content of the lignin, as well as on the hydrogen ion concentration of the buffer. Interpretation of the results was complicated by the fact that sulphonation of the lignin was not uniform. However, he concluded that once the lignin contained a minimum of 6% sulphur, the rate of subsequent cooking was governed by the hydrogen ion concentration of the liquor.

The present investigation does not substantiate the above conclusion. It is difficult to explain the results on the basis of Hägglund's theory, since the authors have shown that the hydrogen ion concentration alone is not the controlling factor in determining the rate of cooking. Yorston (19, 20), from a measurement of the degree of sulphonation at various stages in cooking, concluded that the sulphonation reaction was slow enough to limit to some extent the rate of pulping. He also found that more sulphur was combined with the lignin than was necessary for subsequent cooking in a buffer solution. It is therefore suggested that the sulphonation reaction governs the rate of cooking and is determined by the product of the concentrations of the hydrogen and bisulphite ions, and not by either ion alone. If sulphonation is stopped by replacing the liquor by a buffer solution, naturally the subsequent cooking will be determined by the hydrogen ion concentration of the buffer.

The results reported above indicate the failure of existing theories to explain all the observed facts in regard to sulphite cooking. Possible interpretations have been made, but much more information is needed before the mechanism of the reaction can be completely elucidated. From a practical point of view, a considerable number of data have been provided for a study of sulphite cooking under conditions approaching commercial practice. The effect of various constituents of the liquor on the yield of pulp has been clearly shown, so that the most advantageous conditions may be chosen.

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PASTURE STUDIES XII

THE NATURE OF THE ORGANIC PHOSPHORUS IN SOILS[1]

By C. L. Wrenshall[2] and R. R. McKibbin[3]

Abstract

An improved procedure for the separation of nucleic acid material from soil is described. By its use as much as 65% of the apparent organic phosphorus in soil has been separated in the nucleic acid fraction; this shows that most of the soil organic phosphorus exists in this form. Material of this nature enters the soil solution.

The material separated yielded, on hydrolysis, phosphoric acid, pentose sugar, adenine, and uracil. This indicates that it is a mixture of mononucleotides.

The presence of traces of ether-soluble phosphorus in soil was confirmed.

The presence of organic phosphorus compounds in soil has been established by the finding of ether-soluble phosphorus in low concentration by Aso (1) and by Stoklasa (20), and by the isolation of phosphorus-bearing material of a nucleic acid nature by Shorey (18) and by Bottomley (4). Methods for estimating the organic phosphorus content of soil have been proposed by Potter and Benton (12) and by Schollenberger (16). Analyses made according to these methods, and by other means, have revealed that soils, in general, contain considerable amounts of organic phosphorus compounds (2, 6, 9, 17).

Shorey and Bottomley did not record the yield of phosphatic material obtained, nor its phosphorus content, so that the proportion of the total phosphorus contained therein cannot be calculated from their data. Shorey intimated that his method of isolation was inefficient to the extent that no estimate of the total amount of nucleic acid could be made. Schmoeger (15) and Aso (1) believed that the greater part of the organic phosphorus of soil was of nuclein nature. Schollenberger (16) and Auten (3) failed, however, to obtain evidence of the presence of any considerable quantity of nucleic acid.

To define the organic phosphorus of soils, it is necessary to determine the amounts present in the forms already detected, namely, lecithin and nucleic acid, and to establish the identity and abundance of other organic compounds of phosphorus should such be present in soil. This paper is mainly concerned with the first of these considerations.

Experimental Methods

The method of Potter and Benton (12) was used to estimate the total organic phosphorus in soil. Organic phosphorus in solution was estimated by the method of Parker and Fudge (10). These methods were considered to be sufficiently accurate for the purpose of this study.

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Condensed from a Ph.D. thesis presented to the Faculty of Graduate Studies and Research of McGill University by C. L. Wrenshall.

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Lipoid phosphorus was estimated by exhaustively extracting a sample of soil first with ether and then with absolute ethanol, and determining the total ether-soluble phosphorus in the extracts.

The separation of nucleic acid was effected by a procedure similar in principle to that used by Shorey (18) but modified in the following respects: The soil under study was first extracted with dilute acid so that a maximum amount of phosphorus would then be dissolved by dilute alkali (16, 19). Ammonium hydroxide was used instead of sodium hydroxide for dissolving the organic matter, as the excess could be removed without neutralization. The "humic acid" was reprecipitated to recover the nucleic acid carried down in this fraction (13). A higher concentration of alcohol was necessary to obtain complete precipitation of the nucleic acid. The duration of the treatment was made as short as possible, and care exercised at all stages to minimize hydrolytic destruction. The details of the procedure employed are as follows:

A suitable quantity of soil (100 to 1000 gm.) was extracted with cold *N* hydrochloric acid until the leachings were virtually free from calcium. The soil was washed with distilled water until practically free from acid, and then extracted for 24 hr. by occasional agitation with 10 times its weight of cold 4% ammonium hydroxide. The dark liquor was siphoned from the settled soil to a vacuum distillation apparatus, and the excess ammonia removed by volatilization at a temperature below 40° C. The almost neutral liquid was acidified with 2*N* hydrochloric acid, and the precipitate of "humic acid" allowed to settle. The supernatant liquid was decanted through a free-flowing filter paper, and finally the precipitate was transferred to the filter and washed once with distilled water.

The precipitate was redispersed in the minimum amount of 4% ammonium hydroxide, and the volume was made up with water to that of the original alkaline extract. The excess of ammonia should be slight. After acidifying, decanting, and filtering as before, the acid filtrates were combined. The reprecipitation of the "humic acid" was repeated a second time. In general, further reprecipitation did not lead to an increase in the yield of organic phosphorus.

The acid filtrates were neutralized as obtained, and any precipitate that formed was discarded. The combined filtrates were concentrated by vacuum distillation below 35° C. until further distillation was difficult. The concentrate was acidified with 5% of its volume of concentrated hydrochloric acid, and introduced with vigorous stirring into five times its volume of commercial absolute ethanol. A finely divided, gelatinous precipitate formed immediately, slowly coalesced, and sank to the bottom after several hours. The supernatant liquid was decanted and the precipitate collected on a hardened filter paper, with gentle suction. It was washed with acidified 95% ethanol, and finally with absolute ethanol. The filtrate was free from organic phosphorus, and the precipitate was free from inorganic phosphate. After complete drying in a vacuum desiccator the product was a light-brown amorphous substance that crumbled readily to a powder.

The organic phosphorus-bearing material was examined by the methods outlined by Jones (5) in order to establish its nucleic acid character.

Experimental Data

A sample of typical black muck soil, containing 0.212% of phosphorus in the air-dried state, was examined. The Potter and Benton method of analysis indicated that 55.4% of the total phosphorus was organically combined. Of the total organic phosphorus, 0.31% was shown to be ether-soluble, and 65.0% was present in the nucleic acid precipitate. The latter figure was obtained from the following data: 100 gm. of the soil was extracted with 1200 cc. of 4% ammonium hydroxide, of which one litre was decanted. Following the procedure already described, a yield of 1.12 gm. of impure nucleic acid material was obtained. This material was free from inorganic phosphate but contained 5.67%, or 0.0635 gm., of phosphorus in organic combination. According to the Potter and Benton analysis, 0.0995 gm. of organic phosphorus should have been present in the litre of alkaline extract. On this basis the recovery of organic phosphorus in the nucleic acid precipitate was 65.0%.

A sample of typical brown forest soil (8) contained 0.071% of phosphorus of which 31.2%, according to the Potter and Benton method, was in organic combination. Of the total organic phosphorus 0.25% was shown to be ether-soluble, and 47.5% was present in the nucleic acid precipitate.

The observation of Pierre and Parker (11) that organic phosphorus is a normal constituent of soil water is substantially confirmed here. Table I shows the relative amounts of organic and inorganic phosphorus appearing in water extracts of pasture soils of the brown forest soil type.

TABLE I

Phosphorus in water extracts of soil

Sample designation	Concentration of phosphorus, parts per million of solution		
	Total	Inorganic	Organic
R—untreated	0.225	0.047	0.178
R—fertilized with superphosphate	0.365	0.204	0.161
D—untreated	0.160	0.011	0.149

In other work carried on in this laboratory (14), large quantities of water percolate from podsol raw humus had been concentrated *in vacuo*, and the material insoluble in 70% ethanol separated and dried. Ten grams of this material was dispersed in 200 cc. of 5% hydrochloric acid and poured into one litre of absolute ethanol in the manner described for the separation of nucleic acids. A precipitate formed; it was separated in the usual way. The dry product weighed 0.76 gm. and contained 3.63% of phosphorus in organic combination.

A composite sample of the organic phosphorus-bearing material obtained in a number of experiments was shown to yield, on hydrolysis, the following:

(a) Pentose sugar; demonstrated by evolution of furfural, by positive reactions with phloroglucin and orcin, and by reduction of Fehling's solution.

(b) Phosphoric acid; precipitable as magnesium-ammonium-phosphate or ammonium-phospho-molybdate.

(c) Adenine; isolated as the picrate, clusters of pale yellow needles melting at 271° C.* with decomposition.

(d) Uracil; isolated as colorless needle clusters melting with decomposition at 330° C. after first turning brown at 280° C. This substance would not form a picrate, and responded to the Wheeler and Johnson color reaction for pyrimidines.

No other purine or pyrimidine substances could be demonstrated in the hydrolysate.

Discussion

The hydrolytic products of the organic phosphorus-bearing material separated from soil show its nucleic acid character, and the claims of previous workers in this respect are thus substantiated. The constituents reported here are the same as those obtained by Bottomley (4) on decomposing similar material extracted from English peat. Bottomley concluded that the material was adenine-uracil dinucleotide. However, this conclusion is probably incorrect. Levene and co-workers (7) have demonstrated that the adenine-uracil dinucleotide supposed to result from a decomposition of plant nucleic acids is, in reality, a mixture of two mononucleotides separable by fractional crystallization. Thus the factual existence of an adenine-uracil dinucleotide has never been demonstrated, and its occurrence is held to be most unlikely. The components of the material isolated by Shorey (18) were not the same as those reported here, and varied from soil to soil. In no case have all the components of nucleic acid been detected together in one of these preparations —guanine has never been reported—so that it is most unlikely that unchanged nucleic acid persists to an appreciable extent in soil. Altogether it seems most logical to conclude that the material separated from soil is a mixture of mononucleotides, in the present instance a mixture of adenine nucleotide (adenylic acid) and uracil nucleotide (uridylic acid).

As the figures for the phosphorus content indicate, the material obtained in this study was far from pure. On ignition, a large ash containing some iron remained. Reprecipitation did not result in a pure product, as the phosphorus content was not enhanced, nor were the characteristics of the material improved. In so far as can be judged from their descriptions, the previous preparations of a similar nature were likewise very impure.

**Melting points are uncorrected.*

The data for the percentage yield of organic phosphorus obtained in the ether-soluble and nucleotide fractions indicate that ether-soluble phosphorus is present in very low concentration, while the nucleotide phosphorus is the most abundant form of organic phosphorus present in soil. Additional force is given to the latter statement by the crudeness of the means of estimating the nucleotide phosphorus; unquestionably some of this material was destroyed and lost in the course of separation. The amounts actually present in the soils must be materially greater than those reported. It may very well be that nucleotides are the only organic phosphorus compounds which persist in soil to any considerable extent.

The presence of appreciable quantities of organic phosphorus in water extracts of soils, and the separation of nucleotide phosphorus from a water extract of podsol raw humus, indicate that nucleotides are normal constituents of the soil solution.

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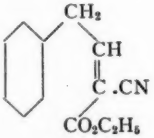
A SYNTHESIS OF 1-HYDROXY-2-NAPHTHOIC NITRILE[1]

BY JOHN A. MCRAE[2] AND LÉO MARION[3]

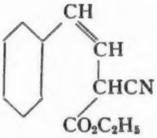
Abstract

The neutral product of the condensation of phenylacetaldehyde with ethyl *sodio*cyanoacetate when distilled under low pressures loses the elements of ethyl alcohol and forms 1-hydroxy-2-naphthoic nitrile. This is identical with the synthetic substance prepared through a series of reactions from α-naphthol. The methyl ether has been prepared from both the synthetic substance and the product of the condensation. 1-Hydroxy-2-naphthoic nitrile can be coupled readily with *p*-nitrobenzenediazonium chloride. The evidence relating to the structure of the neutral condensation product is reviewed.

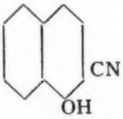
The condensation of phenylacetaldehyde with ethyl cyanoacetate has been investigated by Haworth (3) and later by Linstead and Williams (7). According to the former the reaction produces mainly ethyl α-cyano-α-styrylacetate (II),



I



II



III

together with αα′-dicyano-β-benzylglutaric acid. On the other hand, Linstead and Williams hold that the product consists of a mixture of (II) and ethyl α-cyano-β-benzylacrylate (I) in which the form (II) predominates. These authors make no mention of the substituted glutaric acid, possibly because in most of their work diethylamine was used as the condensing agent instead of the sodium ethoxide used by Haworth.

In the course of a reinvestigation of this reaction it has been found, as already known, that the product consists principally of an ester fraction together with αα′-dicyano-β-benzylglutaric acid. With the exception of the melting point, the properties of this acid have been found to agree with those recorded by Haworth; he gives the melting point as 173° C. while the authors were unable after many recrystallizations to raise the melting point beyond 168° C[*]. Therefore, it was identified further by conversion to β-benzylglutaric acid.

αα′-Dicyano-β-benzylglutaric acid is obtained also when phenylacetaldehyde is shaken with aqueous sodium cyanoacetate. It is then accompanied by two other acidic fractions which can be separated through their barium salts. It was thought that these two fractions might be chiefly the acids corresponding to esters (I) and (II), but it was not found possible to obtain them sufficiently pure for identification.

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Contribution from the Chemical Laboratories, Queen's University, Kingston, and the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

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[*] *All melting points determined by us are corrected.*

The ester fraction can be distilled at 182° C. at 15 mm., but at pressures of 2 to 4 mm. distillation is accompanied by a great deal of frothing, and a crystalline substance is deposited in the condenser. This substance, after recrystallization from benzene, melts at 178° C. It is phenolic in character but otherwise neutral and can be sublimed unchanged at 145° to 150° C. at 1 mm. Its analytical figures are in excellent agreement with $C_{11}H_7ON$, although the molecular weight determined by the Rast method is high (204; calcd. 169). It may be pertinent to mention here that this substance is not identical with that compound (m.p. 220° C.), which Linstead and William, isolated in a condensation catalyzed by sodium ethoxide. In one of our condensations which seemed to behave abnormally, a compound (m.p. 226° C.), apparently identical with that isolated by Linstead and Williams, separated from the ethereal solution of the product during treatment with dilute sodium bicarbonate, but was obtained in amount insufficient for further examination.

The composition of the distillation product, m.p. 178° C., differs by C_2H_6O from that of ester (I) or (II), which, therefore, seems to have been derived from it by loss of ethyl alcohol accompanied by ring closure, and it appears certain that the transformation must have taken place during the heating under low pressure. For this compound the most probable structure appeared to be that of 1-hydroxy-2-naphthoic nitrile (III). Both structures (I) and (II) lend themselves to such ring closure involving simultaneous hydrogen wandering, although it would seem that the transformation might proceed more readily from the $\beta\gamma$-form (II).

Such a reaction is not unique but comparable with the well known synthesis of α-naphthol from β-benzylidene-propionic acid (1). Recently Menon (9) found a strictly analogous case in the production of ethyl 1-hydroxynaphthalene-2, 4-dicarboxylate instead of the expected ethyl γ-carbethoxy-α-phenylglutaconate from the condensation of ethyl phenylacetate and ethyl ethoxymethylmalonate in the presence of sodium ethoxide. Menon (10) has observed lately several other examples using halogenated phenylacetic esters and naphthylacetic ester in place of ethyl phenylacetate. It is noteworthy that in the production of ethyl 1-hydroxynaphthalene-2, 4-dicarboxylate, as in that of 1-hydroxy-2-naphthoic nitrile now placed on record, ring closure takes place during distillation *in vacuo*.

For the complete identification of this reaction product the synthesis of 1-hydroxy-2-naphthoic nitrile was effected. α-Naphthol was converted by the Kolbe reaction into 1-hydroxy-2-naphthoic acid. This on reduction gave rise to 1-hydroxy-2-naphthaldehyde. The oxime of this aldehyde when treated according to Passerini's method (11) was converted smoothly into 1-hydroxy-2-naphthoic nitrile, m.p. 179° C. This melting point was not depressed when this substance was admixed with the condensation product, m.p. 178°C. The methyl ethers prepared from the two substances had the same melting point (50° to 51° C.) either alone or after admixture. An attempt to prepare the benzoates, however, led to abnormal products which, although identical with each other, did not agree in composition with that of the expected benzoate. 1-Hydroxy-2-naphthoic nitrile couples readily with *p*-nitrobenzenediazonium chloride, giving rise to a highly colored product, m.p. 275° C., presumably, 4-*p*-nitrobenzeneazo-1-hydroxy-2-naphthoic nitrile.

Herz and Schulte (4) prepared this nitrile from 2-cyano-1-naphthalenesulphonic acid (m.p. 179° C.). Passerini and Grulis (12) also obtained it as a product of the reaction of mercury fulminate on an alcoholic solution of α-naphthol and potassium cyanide. The melting point given by them (171° to 172° C.) shows, however, that their product was not quite pure.

It seems doubtful if Linstead and Williams have adduced sufficient evidence to warrant their conclusion, which has been accepted by Hugh and Kon (5) that the ester derived from condensing phenylacetaldehyde with ethyl cyanoacetate consists mostly of the βγ-form (II) in equilibrium with some of the αβ-form (I). Aside from the physical evidence of the exaltation of the molecular refractivity, which Linstead and Williams consider is untrustworthy in the present instance, there is little if any chemical evidence to support the βγ-structure. The authors have found that the ester fraction, b.p. 182° C. at 15 mm., reacted readily with potassium cyanide to add on the elements of hydrogen cyanide, and that the product on hydrolysis gave rise to benzylsuccinic acid. This, and the ready reaction with ethyl cyanoacetate and ammonia investigated by Linstead and Williams, support the αβ-structure. The great reluctance of the substance to add ozone, observed by Linstead and Williams, which the authors confirm fully, also supports the αβ-unsaturated formula. In a similar instance it has been shown previously (8) that while the αβ-unsaturated ethyl cyclohexylidene-cyanoacetate reacts very slowly with ozone, ethyl α-*n*-butyl-Δ′-cyclohexenylcyanoacetate and the corresponding ethyl α-*n*-ethyl-Δ′-cyclohexenylcyanoacetate, both of which having the double bond undoubtedly in the βγ-position, react rapidly (2). The production of αα′-dicyano-β-benzylglutaric acid in the reaction itself is likewise indicative of the αβ-phase. The evidence on which Linstead and Williams rely chiefly is the oxidation of the ester to benzoic acid by a solution of chromic acid in acetic acid. They state that under the conditions used by them phenylacetaldehyde is not oxidized to benzoic acid. Repeated efforts have been made to confirm this statement, but in every experiment that the authors performed, following as exactly as possible the conditions used by Linstead and Williams, benzoic acid was obtained as an oxidation product of phenylacetaldehyde.

Thus it may be concluded that the evidence for the predominance of Structure (II) in the product of the condensation of phenylacetaldehyde with ethyl cyanoacetate is little if any stronger than that adduced for maintaining that the product of the condensation of cyclohexanone with ethyl cyanoacetate is ethyl cyclohexenylcyanoacetate (2), rather than the ethyl cyclohexylidenecyanoacetate (6) which it is now generally accepted to be.

Experimental

Condensation of Phenylacetaldehyde with Ethyl Cyanoacetate

A mixture of 12 gm. of phenylacetaldehyde with ethyl *sodio*cyanoacetate prepared from 11.5 gm. of ethyl cyanoacetate and 2.3 gm. of sodium dissolved in 45 cc. of absolute alcohol was refluxed on the steam bath. The product was separated into an acid and a neutral fraction. The acid fraction yielded $\alpha\alpha'$-dicyano-β-benzylglutaric acid which, recrystallized from chloroform-petroleum ether mixture, melts at 168° C. Calcd. for $C_{14}H_{12}O_4N_2$: N, 10.6%: equiv. 272. Found: N, 10.3%; equiv. 273. On hydrolysis this acid gave rise to β-benzyl-glutaric acid, m.p. 101° C. Calcd. for $C_{12}H_{14}O_4$: equiv. 222. Found: equiv. 222.4. The ester distilled at 182° C. at 15 mm. but when distilled under 2 to 4 mm. frothed a great deal, and eventually yielded a small quantity of oil and the crystalline 1-hydroxy-2-naphthoic nitrile which was recrystallized from methanol-benzene, m.p. 178° C. Calcd. for $C_{11}H_7ON$: C, 78.10; H, 4.14; N, 8.28%. Found: C, 77.87, 78.03; H, 4.33, 4.23; N, 8.31, 8.46%.

Formation of Benzylsuccinic Acid from the Ester

To 8 gm. of the ester dissolved in 80 cc. of alcohol was added 5 gm. of potassium cyanide dissolved in 7 cc. of water. After standing two weeks in a dark place the product was hydrolyzed at 0° C. with fuming hydrochloric acid, and it yielded 2.8 gm. of benzylsuccinic acid obtained from hot water as glistening plates, m.p. 162° C. Calcd. for $C_{11}H_{12}O_4$: C, 63.46; H, 5.77%; equiv. 208. Found: C, 63.30; H, 5.74%; equiv. 210.

Synthesis of 1-Hydroxy-2-naphthoic Nitrile

1-Naphthol-2-carboxylic acid was prepared by Kolbe's reaction by leading a strong current of carbon dioxide for eight hours into a mixture of α-naphthol and sodium in boiling toluene. The purified acid, m.p. 203° C., was reduced with sodium amalgam to the corresponding aldehyde which was immediately converted into the oxime, m.p. 145° C. (Yield 43%, based on acid used, deduction being made of the recovered acid.) The oxime was then converted into the nitrile by Passerini's reaction (11) as follows: 4 gm. of 1-hydroxy-2-naphthaldoxime and 1.8 gm. of powdered potassium cyanide were dissolved in 14 cc. of alcohol and 4.8 cc. of water, and the solution was refluxed on the steam bath for five hours. The alcohol was then evaporated, water added, and the solution filtered through charcoal. The filtrate was acidified with dilute sulphuric acid and the precipitated nitrile filtered, washed with water, dried, and sublimed at 160° to 170° C. at 0.5 to 1 mm. It was recrystallized (charcoal) from methanol-benzene; prisms, m.p. 179° C., wt. 2.1 gm. Yield, 58.3%. Calcd. for $C_{11}H_7ON$: C, 78.10; H, 4.14; N, 8.28%. Found: C, 78.29, 78.30; H, 4.38, 4.46; N, 8.24, 8.50%.

Methylation of 1-Hydroxy-2-naphthoic Nitrile

Some of the nitrile obtained from the condensation product (0.05 gm.) was dissolved in ether (20 cc.) containing 1 cc. of methanol, and treated at room temperature for 16 hr. with an ethereal solution of diazomethane. The excess reagent was evaporated and the product in ether solution was washed with dilute sodium hydroxide, dried, and eventually crystallized from benzene-petroleum ether; long needles, m.p. 50° to 51° C. Calcd. for $C_{12}H_9ON$: OCH_3, 16.94%. Found: OCH_3, 16.98%. The methyl ether was also prepared in the same way from the synthetic product, m.p. 49° C. Found: OCH_3, 16.73%. Admixture of the two methylated nitriles did not depress the melting point (m.p. 50° to 51° C.).

Benzoylation of 1-Hydroxy-2-naphthoic Nitrile

This reaction followed an anomalous course. 1-Hydroxy-2-naphthoic nitrile (from condensation), 0.1 gm., was dissolved in 3 cc. of pyridine, and the solution was warmed on the steam bath for one hour with 0.7 cc. of benzoyl chloride, and then allowed to stand at room temperature for some time. The product was worked up in the usual way and crystallized (charcoal) from benzene, m.p. 159° to 160° C. Calcd. for $C_{18}H_{11}O_2N$: N, 5.13%. Found: N, 7.21, 7.34%. The synthetic nitrile was also treated with benzoyl chloride in pyridine in the same way, m.p. 159° to 160° C. Found: N, 7.16, 7.15%. Admixture of the two products of benzoylation did not depress the melting point; the two, therefore, appear to be identical but do not agree in composition with the expected benzoate.

4-p-Nitrobenzeneazo-1-hydroxy-2-naphthoic Nitrile

1-Hydroxy-2-naphthoic nitrile (0.5 gm.) was dissolved in potassium hydroxide solution and coupled with *p*-nitrobenzenediazonium chloride prepared from *p*-nitraniline (0.3 gm.), sodium nitrite (0.2 gm.), and 1.5 cc. of hydrochloric acid. The azo compound was crystallized from nitrobenzene from which it separated as reddish flakes with a greenish cast, m.p. 275° C. Calcd. for $C_{17}H_{10}O_3N_4$: C, 64.15; H, 3.14; N, 17.61%. Found: C, 64.45, 64.48; H, 3.41, 3.45; N, 17.61, 17.79%.

Condensation of Phenylacetaldehyde with Sodium Cyanoacetate

To a solution of sodium cyanoacetate prepared from 25 gm. of monochloracetic acid were added 15 gm. of phenylacetaldehyde, 15 cc. of alcohol, and 30 cc. of strong sodium hydroxide solution. The mixture was shaken vigorously for five minutes and allowed to stand. The reaction yielded 15.3 gm. of an acidic oil from which $\alpha\alpha'$-dicyano-β-benzylglutaric acid was separated through its soluble copper salt. The remaining oil recovered from the insoluble copper salt contained two acids which were separated as their barium salts, one being insoluble, the other soluble, in water. The acids liberated from their barium salts failed to produce 1-hydroxy-2-naphthoic nitrile when distilled *in vacuo*.

Oxidation of Phenylacetaldehyde

A solution of phenylacetaldehyde (1 gm.) in 5 cc. of glacial acetic acid was treated with 4 gm. of chromic acid in 15 cc. of glacial acetic acid. After the vigorous reaction had subsided the mixture was heated for half an hour and then poured into 200 cc. of water. The solution was extracted with ether and the extract washed free of acetic acid with water. The residue left after distillation of the ether was boiled with a little water, and the solution, decanted from some insoluble oil, yielded shiny white plates, m.p. 120°C. The product did not depress the melting point of a sample of pure benzoic acid (m.p. 122° C.) when admixed with it.

Ozonization of Neutral Condensation Product

Through a solution in chloroform of 2 gm. of the neutral product of the condensation of phenylacetaldehyde with ethyl cyanoacetate a stream of ozonized oxygen was passed for 85 hr. The ozonized substance was then freed of chloroform and treated on the water bath with 25 cc. of 6% sulphuric acid and 3 gm. of potassium ferrocyanide. This yielded a neutral fraction, which, when treated with semioxamazide, failed to disclose the presence of an aldehyde.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

LIII. STRUCTURE OF THE DEXTRAN SYNTHESIZED BY THE ACTION OF LEUCONOSTOC MESENTERIOIDES ON SUCROSE[1]

By Frances L. Fowler[2], Irene K. Buckland[2], Fritz Brauns[3], and Harold Hibbert[4]

Abstract

The dextran synthesized from sucrose by the action of one strain of *Leuconostoc mesenterioides* has been subjected to chemical investigation. The polysaccharide has been hydrolyzed to glucose. The triacetate, tribenzoate, and trimethyl derivative (all calculated on a glucose anhydride unit) have been prepared. Hydrolysis of trimethyl dextran by the action of methanol-hydrochloric acid yielded dimethyl, trimethyl, and tetramethyl methyl glucosides in the ratio of 1 : 3 : 1. The products of hydrolysis have been identified as 2,3-dimethyl methyl glucoside, 2,3,4-trimethyl methyl glucoside, and 2,3,4,6-tetramethyl methyl glucoside.

The results indicate that dextran is a polymer of a pentaglucopyranose anhydride. One of the glucopyranose units is attached as a side chain, the remaining four being most probably connected in linear chain union. Three of the linkages between building units are of the 1,6 type, while the remaining two are either 1,4 or 1,6.

The antigenic properties shown by dextran are probably a result of the presence of the glucose side chains.

Introduction

The present investigation represents one of a systematic series of studies begun several years ago by Hibbert and co-workers on the polysaccharides elaborated by certain species of bacteria. Previous communications (6, 9, 10, 11, 16, 23) have dealt with the synthesis and structure of bacterial levans and celluloses. Furthermore, the nature of the synthesis of dextran from sucrose by the action of *Leuconostoc mesenterioides* was investigated by Tarr and Hibbert (24). As noted by these authors, the occurrence of dextran has been reported in connection with the wine and sugar industries since the time of Pasteur. It was identified by Scheibler (19, 20) as an anhydride of dextrose, and shown by Jubert (13) to be a product of fermentation of sugar solutions.

In recent years the polysaccharides elaborated by bacteria have assumed a position of great importance in the field of immuno-chemistry. The numerous researches of Avery, Heidelberger, and many others have shown that specific polysaccharides are responsible for type specificity and virulence of many

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pathogenic bacteria, and considerable advances have been made in the correlation of specificity with chemical structure.

Preliminary investigations by FitzGerald (5) and by Zozaya (26, 27, 28) have indicated that dextran possesses a certain degree of immunological specificity. Its structure, therefore, becomes a problem of interest, not only to the carbohydrate chemist, but also to the immunologist.

Previous chemical investigations relating to the structure of dextran have been very limited in scope. As mentioned above, Scheibler (19, 20) showed that it was an anhydride of glucose, and his results were confirmed by Jubert (13) and later by Browne (2). Däumichen (4) found that the product formed a triacetate and a tribenzoate; this indicated the presence of three free hydroxyl groups for each glucose-anhydride unit in the molecule.

Up to the present, however, the manner of linkage of the glucose anhydride units with each other has not been determined. In 1931, Tarr and Hibbert (24) intimated that the structure of the polysaccharide was being investigated in these laboratories. The problem has, however, proved difficult of solution, owing in part to the complex nature of the structure, and in part to the great difficulty encountered in effecting a complete methylation of the product.

Discussion of Results

The dextran under consideration was produced by the action on sucrose of *Leuconostoc mesenterioides*, "Culture 4", as described by Tarr and Hibbert (24).

When purified by electrodialysis the product was obtained as a pure white powder, free from nitrogen, and containing only 0.07% ash. The empirical formula, determined by combustion analyses, was $(C_6H_{10}O_5)_x$.

Hydrolysis of dextran with dilute sulphuric acid gave a 90% yield of glucose. The formation of a triacetate and a tribenzoate (calculated on a glucose-anhydride unit) was confirmed.

Methylation by means of dimethyl sulphate and alkali, as well as silver oxide and methyl iodide, resulted in a methoxyl content of only 40 to 41% (theoretical 45.6%). This value was increased to 43.3% by the use of thallium ethylate and methyl iodide, but the polysaccharide proved extremely resistant to further methylation.

A methoxyl content of 45.4% was finally obtained by a slight modification of the method of Muskat (17,18), which consists in first forming the sodium or potassium derivative in liquid ammonia, followed by treatment with a methylating agent such as methyl iodide.

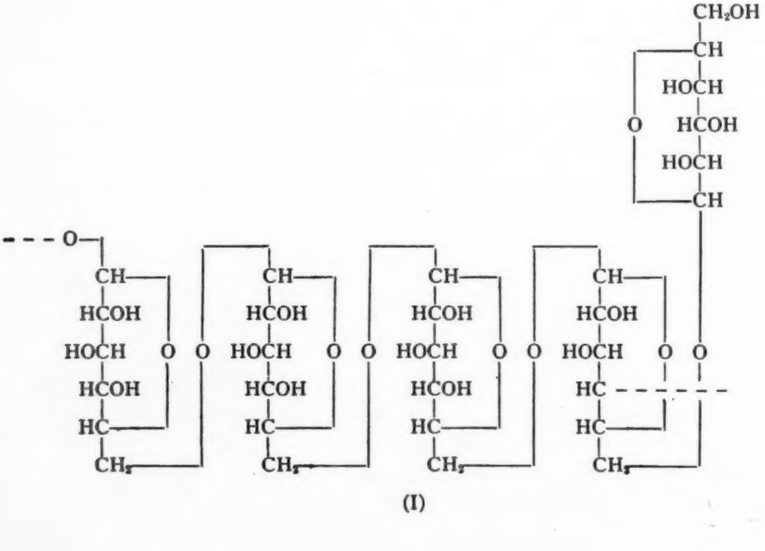
The completely methylated product, on hydrolysis with hydrogen chloride in absolute methanol, yielded, on fractionation, a mixture of dimethyl, trimethyl, and tetramethyl methyl glucosides in the ratio of 1 : 3 : 1. The tetramethyl methyl glucoside, on hydrolysis, yielded crystalline 2,3,4,6-tetramethyl glucose. This showed that this unit is present in the pyranose form in the original polysaccharide, and that it is linked to the rest of the molecule through carbon atom 1. The latter fact finds confirmation in the non-reducing character of the dextran, which indicates that all the reducing groups in the building units are chemically linked.

From the trimethyl fraction, crystalline 2,3,4-trimethyl-β-methyl glucoside was isolated. This proved that the hydroxyl groups in positions 2, 3, and 4 are free in the original polysaccharide, and that the original ring structure is of the pyranose form.

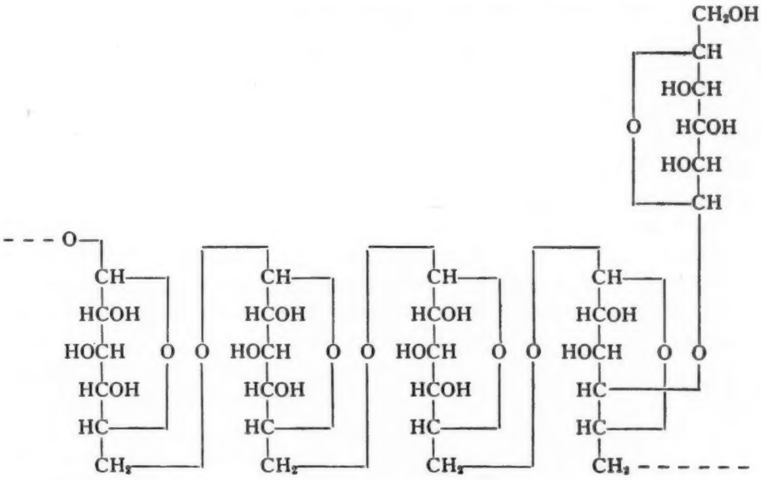
Treatment of the dimethyl methyl glucoside with trityl chloride yielded crystalline 2,3-dimethyl-6-trityl-α-methyl glucoside. In this fraction, therefore, the hydroxyl groups on carbon atoms 2 and 3 were free in the original polysaccharide. No conclusions can be drawn regarding the ring structure of this unit, since, although the glucoside is of the pyranose form when isolated, the possibility of ring shift from carbon atom 4 to carbon atom 5 during hydrolysis has not been excluded in this case. However, because the pyranose form has been shown to be present in the other units of the polysaccharide, and since no naturally occurring glucofuranose compounds are known, a uniform glucopyranose structure for all units seems highly probable.

On the basis of the evidence cited above, two tentative formulas for dextran, differing only in the position of attachment of the side chain, may be put forward.

It can readily be seen, however, that Formulas I and II do not exhaust all the possibilities for the structure of dextran on the basis of the present experimental evidence. It is possible that the side chain may consist of two, three, or even four units, with a corresponding shortening of the primary chains.

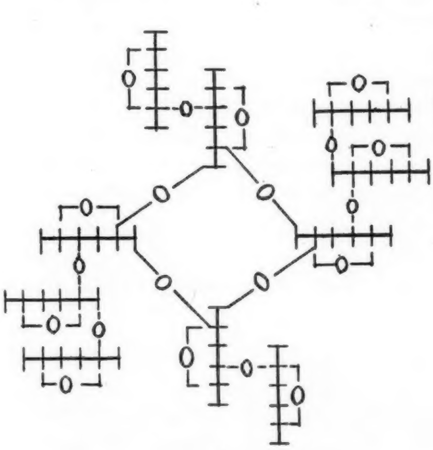


(I)

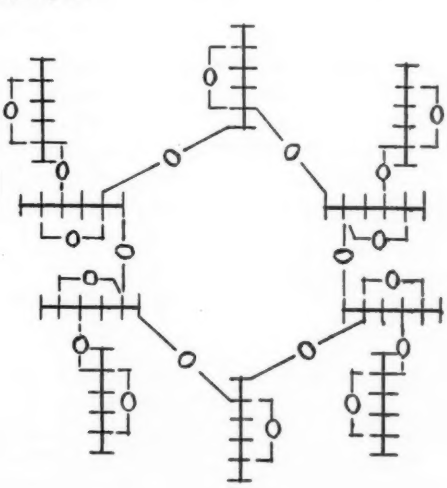


(II)

The same problem is presented in the case of the recently investigated polysaccharide, graminin. The formula suggested by Schlubach and Koenig (21) for this product is the ring structure, *A*.



A



B

This formula was deduced from the facts that the polysaccharide is non-reducing, has a molecular weight corresponding to 10 fructose units, and, on methylation and hydrolysis yields dimethyl, trimethyl, and tetramethyl methyl fructosides in the ratio 2 : 1 : 2. It is evident, however, that the larger ring, *B*, in which the side chains of only *one* unit are all similar, would explain the facts equally well.

Antigenic Properties of Dextran

Recent investigations on the bacterial polysaccharides have indicated the importance of their specific immunological role (pneumococcus, etc.), and the fact that dextran has a marked antigenic character would seem to point to this latter action as due to the presence therein of the glucose side chain.

In this connection it is of interest that a yeast polysaccharide shown previously by Kesten, Cook, Mott, and Jobling (14) to have antigenic properties has now been isolated by Sevag, Cattanio, and Mauweg (22). It has an acetyl content of about 9% and is precipitated by the antiserum of Type 2 pneumococcus (0.01% solution). Furthermore, Haworth, Hirst, and Isherwood (7) found that the yeast polysaccharide isolated by them by alkaline extraction also contains side chains of a hexose unit.

The antigenic action shown by dextran was found by FitzGerald and co-workers to disappear when the nitrogen content fell below 0.2%. In view, however, of the decisive antigenic role played by a small percentage of acetyl in certain polysaccharides, as shown quite recently by Avery and Goebel (1), it is possible that the negative action found by FitzGerald and co-workers (5) may be due to the removal of acetyl groups arising in the final purification of the dextran carried out in these laboratories.

These questions are now the subject of investigation by E. C. Fairhead.

Experimental

Purification of Dextran by Electrodialysis

Dextran (20 gm.) prepared according to the method of Tarr and Hibbert (24) was dissolved in 500 cc. of 0.4 *N* sodium hydroxide solution, and electrodialyzed (potential, 110 volts) in the Pauly apparatus for one week against distilled water. The solution was then poured in a fine stream, with vigorous mechanical stirring, into three times its volume of anhydrous methanol.

The resulting flocculent white precipitate was free from reducing sugars and nitrogen. Ash, 0.07%. $\alpha_D = +198.3°$ in 1.117 *N* sodium hydroxide solution, $c = 1.86\%$. Found (microanalysis): C, 44.6; H, 6.30%. Calcd. for $(C_6H_{10}O_5)_x$: C, 44.5; H, 6.18%.

Hydrolysis of Dextran with Dilute Sulphuric Acid

Dextran (1.0 gm.) was dissolved in 15 cc. of water and 10 cc. of 70% sulphuric acid was added slowly, while cooling with ice. After standing for four days at 36° C. the solution was clear and colorless. It was then diluted to 100 cc. and heated in a boiling-water bath. After six hours the rotation was constant. The solution was neutralized with barium carbonate, decolorized with charcoal, filtered, evaporated under reduced pressure to a thick syrup, and the syrup dissolved in hot absolute ethanol. On cooling, 1.0 gm. of white crystals was obtained, which, on further recrystallization from ethanol, gave $\alpha_D = +51.2°$. The product formed an osazone; m.p. 204.5 to 205° C. *

**Melting points are not corrected.*

In order to obtain a quantitative estimation of the yield of glucose, the method of Willstätter and Schudel (25) was employed. By this method, dextran (0.5688 gm.) gave, on hydrolysis, a 90.5% yield of glucose (0.5710 gm.).

Benzoylation of Dextran

Anhydrous dextran (1 gm.) was dissolved in 19 cc. of dry quinoline and 4.5 cc. of benzoyl chloride added. The mixture was heated for three hours at 120° to 130° C., whereupon there was formed a thick gel, which solidified on cooling to a light-brown mass. This was dissolved in chloroform, and the solution extracted successively with water, 5% sulphuric acid, water, saturated sodium bicarbonate solution, and again with water. The pale red solution was dried over sodium sulphate, then centrifuged and evaporated under reduced pressure to a volume of 50 cc. It was poured slowly, with stirring, into absolute methanol, and the resulting finely divided white precipitate washed with absolute methanol, then with petroleum ether, and vacuum dried over sulphuric acid and potassium hydroxide. $\alpha_D = +193.7°$ in acetylene tetrachloride, $c = 2.44\%$. Found: C_6H_5CO, 64.67%. Calcd. for $C_6H_7O_5(C_6H_5CO)_3$: C_6H_5CO, 66.45%.

Acetylation of Dextran

Preliminary experiments showed that dextran does not dissolve in dry pyridine at 80° C., so that use of aqueous pyridine solution was necessary.

Dextran (2 gm.) was suspended in 20 cc. of pyridine containing 4 cc. of water and stirred vigorously for several hours. Approximately half the dextran appeared to dissolve. Further quantities of pyridine (25 cc.) and acetic anhydride (33 cc.) were added with continuous stirring over a period of three hours at room temperature. The mixture was then stirred and heated for four hours at 50° C. and one hour at 80° C. The insoluble product was removed by centrifuging, and the supernatant liquor poured on to cracked ice. Only a very flocculent precipitate formed. The product remaining in the centrifuge jar was washed with water and vacuum dried. Yield, 2.7 gm. (79%). Found: CH_3CO, 44.1%. Calcd. for $C_6H_7O_5(CH_3CO)_3$: CH_3CO, 44.8%.

*Methylation of Dextran**

Dextran (24 gm.) was methylated with 80 cc. of dimethyl sulphate and 196 cc. of 50% potassium hydroxide in the usual manner, and the resulting solution dialyzed until free from sulphates. A small portion was poured into acetone and the precipitated product separated and analyzed (OCH_3, 12.8%). The methoxyl content was increased to 30.7% by a second methylation.

The precipitated dextran was now dissolved in 200 cc. of dry anisol**, and the solution frozen by immersion of the reaction flask in a mixture of dry-ice and acetone at −50° C. Dry ammonia (100 cc.) was condensed on the frozen mass and 3 gm. of sodium dissolved in it. The temperature of the bath was raised to −35° C., which is between the melting point of anisol and the boiling point of ammonia. After the anisol solution had melted it was shaken thoroughly with the ammonia solution. The ammonia was then allowed to evaporate, the last traces being removed by distilling off a portion of the anisol under reduced pressure.

**Procedures involving the use of silver oxide and methyl iodide, and of thallium ethylate and methyl iodide which resulted in incomplete methylation of other samples of dextran are not described.*

***The application of this solvent, which has been used in this laboratory for the last few years, has recently been described by Hess (8).*

Methyl iodide (15 cc.) was added, and the solution refluxed overnight at 40° C. After removal of the anisol by distillation, the residue was dissolved in approximately 500 cc. of water and dialyzed. After removal of the water, the product was dissolved in chloroform and precipitated in low-boiling petroleum ether. The methoxyl content was now 41.9%. After a total of five methylations by this method the methoxyl content was 45.4%. $[\alpha]_D^{21°} = +202.2°$ in acetylene tetrachloride, $c = 0.758\%$. Found (microanalysis); C, 52.81; H, 7.73; OCH_3*, 45.4%. Calcd. for $C_6H_7O_2(OCH_3)_3$, C, 53.0: H, 7.85; OCH_3, 45.6%.

Hydrolysis of Fully Methylated Dextran

Fully methylated dextran (12.0 gm.) was heated in a sealed tube with a solution of 1.5% by weight of anhydrous hydrogen chloride in 150 cc. of absolute methanol. The heating was carried out in a tilting-oven, for 24 hr. at 125° C., then for 24 hr. at 145° to 150° C. After this time the dextran had completely dissolved, and the solution was clear and light-yellow.

The product was freed from hydrochloric acid by shaking it for two hours with 30 gm. of silver carbonate. After the solution had been filtered through charcoal it was shaken for 12 hr. with charcoal, to remove traces of silver salts, and filtered.

The clear, colorless solution was evaporated at a pressure of 15 mm. to a pale yellow syrup; weight, 12.05 gm. (87% of the calculated yield). The syrup did not reduce Fehling's solution. Its methoxyl content corresponded to that of trimethyl methyl glucoside. Found (microanalysis): OCH_3, 52.2%. Calcd. for $C_6H_8O_2(OCH_3)_4$: OCH_3, 52.6%.

The syrup distilled between 75° and 125° C. (pressure 0.01 mm., bath temperature, 95° to 170° C.). The first portion of the distillate, a colorless mobile oil, was followed by a viscous pale yellow syrup. A methoxyl determination on the last portion of the distillate gave a value corresponding to dimethyl methyl glucoside. $OCH_3 = 42.1\%$. Calcd. for $C_6H_9O_3(OCH_3)_3$: 41.9%. Since the methoxyl content of the original mixture was that of a trimethyl methyl glucoside, it is apparent that dimethyl and tetramethyl methyl glucosides were present in equivalent amounts.

Separation of the Hydrolysis Products of Fully Methylated Dextran

A portion (7.5 gm.) of the original hydrolysis products (OCH_3, 52.2%) was dissolved in 100 cc. of water, and the solution exhaustively extracted with chloroform according to the procedure of Macdonald (15). The aqueous

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**All analyses for methoxyl content were made by the Vieböck and Schwappach method as described by Clark (3).*

TABLE I

PRODUCTS OF HYDROLYSIS OF FULLY METHYLATED DEXTRAN

Product	Yield from 7.5 gm. of hydrolytic products, gm.	Corrected yield, gm.	Calcd. for 1 : 1 : 1 ratio		Calcd. for 1 : 2 : 1 ratio		Calcd. for 1 : 3 : 1 ratio		Calcd. for 1 : 4 : 1 ratio	
			Theor. yield, gm.	Yield, %	Theor. yield, gm.	Yield, %	Theor. yield, gm.	Yield, %	Theor. yield, gm.	Yield, %
Dimethyl methyl glucoside (mol. wt. 222.15)	1.40	1.70	2.35	59.6	1.76	79.5	1.41	99.3	1.18	119.0
Trimethyl methyl glucoside (mol. wt. 236.16)	3.57	4.34	2.50	141.0	3.75	94.0	4.50	78.3	5.0	70.5
Tetramethyl methyl glucoside (mol. wt. 250.18)	1.20	1.17	2.65	45.3	1.99	60.3	1.59	75.5	1.32	91.0

solution (containing the dimethyl methyl glucoside), on evaporation under reduced pressure, yielded a light-brown, viscous syrup. Yield, 1.35 gm. The separation of the glucosides constituting the chloroform soluble fraction was carried out by fractional distillation under high vacuum, as shown schematically in Fig. 1. All fractions were controlled by determinations of refractive index, in order to obviate the possibility of contamination of the trimethyl fraction with equal parts of the dimethyl and tetramethyl derivatives.

From the methoxyl contents of the various fractions, it is evident that fractions V*a* and V*b* consist essentially of tetramethyl methyl glucoside, IV*c*, and IV*d* of trimethyl methyl glucoside, and II*f* and IV*e* of dimethyl methyl glucoside. The total amount of trimethyl methyl glucoside from the original 7.5 gm. of hydrolytic products was 3.57 gm. (made up of 2.03 gm. of fractions IV*c*, IV*d*, V*c*, and 1.54 gm. of crystalline material isolated from fractions II*b* and II*c*), equivalent to a yield of 47.6%. The yields of dimethyl and tetramethyl methyl glucosides (Fractions II*f*+IV*e* and V*a*+V*b* respectively) were 1.40 and 1.20 gm., equivalent to 18.7 and 16% respectively. The working-loss thus amounts to $7.5-6.17=1.33$ gm. or 17.3%. If this loss, in accordance with accepted experimental practice, is allocated *pro rata* to the three fractions, the corrected yields are 1.70, 4.34, and 1.47 gm. respectively. This clearly identifies dextran as the 1 : 3 : 1 polymer.

This ratio of dimethyl methyl glucoside : trimethyl methyl glucoside : tetramethyl methyl glucoside was confirmed by the following experiment.

Check on the Yield of Trimethyl Methyl Glucoside by Hydrolysis to Trimethyl Glucose

A portion of the original glucoside hydrolytic products (4.55 gm.) was dissolved in 100 cc. of water, and the trimethyl and tetramethyl methyl glucosides were removed by chloroform extraction as before. The chloroform solution, on evaporation, yielded a pale yellow syrup. $n_D=1.455$, corresponding approximately to a mixture of 25% of tetramethyl and 75% of trimethyl methyl glucosides. Yield, 3.85 gm.

This syrup was dissolved in 250 cc. of 5% aqueous hydrochloric acid (according to the procedure of Irvine and Oldham (12)). The mixture was heated under reflux on a water bath at 90° to 95° C. for nine hours, after which time the rotation was constant.

The acid solution was extracted five times with 125-cc. portions of chloroform. The combined extracts, on evaporation, yielded 0.575 gm. of a light-yellow syrup, which crystallized on inoculation with a crystal of 2,3,4,6-tetramethyl glucose.

The aqueous solution was neutralized by shaking with silver carbonate, filtered, and shaken overnight with charcoal. On filtration and evaporation under reduced pressure, the solution yielded a viscous syrup that was dark in color owing to the presence of silver salts. Yield, 2.38 gm.; OCH_3, 38.2%. Since the methoxyl content of trimethyl glucose is 41.9%, the actual yield

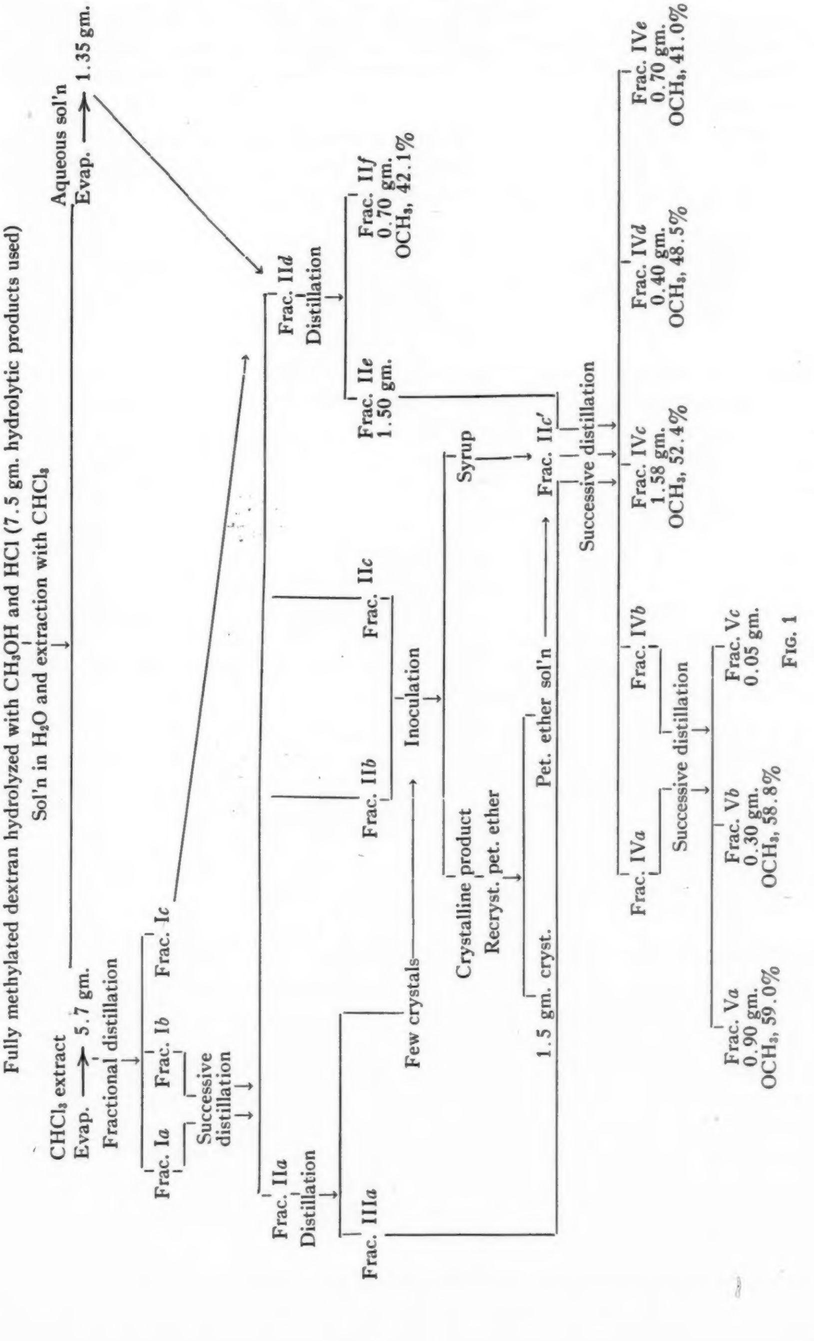
Fully methylated dextran hydrolyzed with CH₃OH and HCl (7.5 gm. hydrolytic products used)
Sol'n in H₂O and extraction with CHCl₃
CHCl₃ extract
Evap. → 5.7 gm.
Fractional distillation
Aqueous sol'n
Evap. → 1.35 gm.
Frac. Ia
Frac. Ib
Frac. Ic
Successive distillation
Frac. IIa
Frac. IIb
Frac. IIc
Frac. IId
Distillation
Frac. IIIa
Few crystals
Inoculation
Crystalline product
Syrup
Recryst. pet. ether
1.5 gm. cryst.
Pet. ether sol'n
Frac. IIe
1.50 gm.
Frac. IIf
0.70 gm.
OCH₃, 42.1%
Frac. IIc'
Successive distillation
Frac. IVa
Frac. IVb
Frac. IVc
1.58 gm.
OCH₃, 52.4%
Frac. IVd
0.40 gm.
OCH₃, 48.5%
Frac. IVe
0.70 gm.
OCH₃, 41.0%
Successive distillation
Frac. Va
0.90 gm.
OCH₃, 59.0%
Frac. Vb
0.30 gm.
OCH₃, 58.8%
Frac. Vc
0.05 gm.

FIG. 1

of the sugar calculated on the basis of methoxyl content is 93% of the 2.38 gm. obtained. This product contained no dimethyl glucose, since shaking in acetone solution with charcoal, filtering, and evaporating yielded a thick colorless syrup: OCH_3, 41.4%.

The yield of 2.38 gm. of crude product, of 93% purity, thus corresponds to 2.21 gm. of pure trimethyl glucose, or 2.35 gm. of trimethyl methyl glucoside, equivalent to 52.3% of the original mixture.

The theoretical yield, calculated on a 1 : 3 : 1 basis is 60%, whereas on a 1 : 2 : 1 basis it is only 50%. The yield obtained thus represents 87% of the theoretical yield for trimethyl methyl glucoside represented in the 1 : 3 : 1 ratio, and is in excess of that required by a 1 : 2 : 1 ratio.

Identification of the Dimethyl Methyl Glucoside

The dimethyl methyl glucoside obtained in fraction IV*e* (Fig. 1) was redistilled, and obtained as a colorless viscous syrup, b.p. 100° to 105° C. (pressure, 0.003 mm.; bath temperature, 125° to 130° C.). Found (by microanalysis: C, 49.9; H, 8.09; OCH_3, 41.4%. Calcd. for $C_6H_9O_3(OCH_3)_2$: C, 48.8; H, 8.12; OCH_3, 41.9%.

A portion (0.5 gm.) of the above product and 0.63 gm. of trityl chloride were dissolved in 4 cc. of dry pyridine and heated for one hour in a boiling water bath. The solution was cooled, the precipitated pyridine hydrochloride removed by filtration, and the filtrate then poured, with stirring, into 50 cc. of ice-water whereupon a pale yellow sticky mass was precipitated. This solidified on standing overnight in the ice box.

The resulting granular precipitate was filtered, washed, and vacuum dried over phosphorus pentoxide at 100° C. After two crystallizations from absolute ethanol, it was obtained in the form of rhombic plates, m.p. 168.5° to 169.5° C. (corrected). The mixed melting-point with a synthetic specimen of 2,3-dimethyl-6-trityl-α-methyl glucoside was 167.5° to 169.5° C. Found (by microanalysis): C, 72.4; H, 7.05; OCH_3, 20.3%. Calcd. for $C_6H_8O_2(OCH_3)_3 . OC(C_6H_5)_3$: C, 72.5; H, 6.91; OCH_3, 20.0%.

Identification of the Trimethyl Methyl Glucoside

During the fractional distillation of the products of hydrolysis, approximately one-half of the trimethyl methyl glucoside was obtained in crystalline form. After the crystals had been pressed on a porous plate and recrystallized three times from low-boiling petroleum ether they were obtained as a mass of white needles. The product was then further purified three times by sublimation at 80° C. at 15 mm. M.p., 94° C. The mixed melting point with a specimen of 2,3,4-trimethyl-β-methyl glucoside* was 94° C. $[\alpha]_D^{20°} = -20.57°$ in chloroform, $c = 3.325\%$. Found (microanalysis): C, 50.46; H, 8.35; OCH_3, 52.0%. Calcd. for $C_6H_8O_2(OCH_3)_4$: C, 50.9; H, 8.49; OCH_3, 52.6%.

**Received through the kindness of Sir James Irvine of the University of St. Andrews.*

Identification of the Tetramethyl Methyl Glucoside

A portion (0.5 gm.) of Fraction V*a* (Fig. 1) was dissolved in 50 cc. of 5% aqueous hydrochloric acid and heated on a water bath at 90° to 95° C. After four hours the rotation was constant.

The acid solution was extracted with five 25 cc. portions of chloroform, and the combined chloroform extracts were evaporated under reduced pressure to a pale yellow syrup. On inoculation with a crystal of 2,3,4,6-tetramethyl glucose, the syrup crystallized.

The product was recrystallized three times from low boiling petroleum ether containing a trace of ether, and obtained as a mass of fine white needles; m.p., 86° to 88° C. When mixed with a specimen of 2,3,4,6-tetramethyl glucose (prepared by hydrolysis of octamethyl maltose) melting at 88° to 90° C., the mixture melted at 86° to 89° C. $[\alpha]_D^{21°} = +105.4°$ in chloroform, $c = 0.587\%$. No mutarotation was observed in the course of 36 hr. Found (microanalysis): C, 50.8; H, 8.41; OCH_3, 51.9%. Calcd. for $C_6H_8O_2(OCH_3)_4$. C, 50.9; H, 8.49; OCH_3, 52.6%.

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